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

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SUPPLEMENTARY MATERIAL

TABLE OF CONTENT

Power calculation
To obtain an effect size of d=0.4 with a power of 80%, we required 60 ADHD adults to obtain 20 participants (a third of 60) that did not respond to treatment [1].

Sample characteristics
60 male adults with ADHD completed the study. They mostly identified as White British (71%), had a mean (± 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 [2].

Table S1. Whole brain morphometric measures.
This table reports total intracranial volume (ICV), mean cortical thickness (CT), and mean surface area (SA) in controls and ADHD responders and non-responders.

We carried out independent-sample t-tests (two tailed) and observed no statistically significant differences in total intracranial volume, mean CT, or mean SA between controls and ADHD individuals (p>.05). However, ADHD treatment non-responders showed statistically significant decreases in *ICV* and mean SA as compared to ADHD treatment responders. No statistically significant difference in mean CT was observed between non-responders and responders. *Significant results are highlighted in bold*.

	Controls	Responders	Non-responders	Re vs Nre
Total ICV (L)	1.55 (1.29)	1.59 (1.12)	1.59 (1.12)	**$t_{(58)}$=2.51, p=.015**
Mean CT (mm)	2.47 (0.07)	2.42 (0.10)	2.43 (0.10)	$t_{(58)}$=0.542, p=.589
Mean SA	112657.739 (8728.84862)	114505.250 (7640.98990)	114505.250 (7640.98990)	**$t_{(58)}$=3.146, p=.003**

Table S2. Subgroup comparisons for clusters significantly different between ADHD and controls.
This table reports the results of the independent-sample t-tests *(two tailed)* comparing morphometric measures between controls and (separately) non-responders and responders, and between these two treatment response groups, for clusters found significantly different between the whole ADHD group and controls. Significant results are highlighted in bold.

As shown, both responders and non-responders significantly differed from controls in these 5 clusters. The two ADHD groups significantly differed from each other only in the cortical volume of cluster 3. Results are displayed in Fig.2.

			Nre	**Re**	**Con**	**Nre vs Con**	**Re vs Con**	**Re vs Nre**
Measure	Cluster	Region Labels	mean (SD)	mean (SD)	mean (SD)	t-test	t-test	t-test
Cortical Volume								
	1	posterior-cingulate cortex, precuneus, superior parietal cortex	1.333(.165)	1.373(.158)	1.503(.156)	**t(39)=-3.359 p=.002**	**t(63)=-3.189 p=.002**	t(58)=-.872 p=.387
	2	pars triangularis, rostral middle frontal gyrus, superior frontal gyrus	2.134(.284)	2.243(.249)	2.390(.273)	**t(39)=-2.918 p=.006**	**t(63)=-2.194 p=.032**	t(58)=-1.484 p=.143
	3	fusiform gyrus, inferior and middle temporal gyri	2.396(.290)	2.589(.294)	2.779(.355)	**t(39)=-3.696 p<.001**	**t(63)=-2.305 p=.012**	**t(58)=-2.337 p.023**
Cortical Thickness								
	1	fusiform gyrus, temporal pole, inferior, middle and superior temporal gyri	2.922(.210)	2.928(.149)	3.079(.114)	**t(39)=-3.040 p=.004**	**t(63)=-4.223 p<.001**	t(58)=-.112 p=.911
	2	supramarginal gyrus, inferior and superior parietal cortices	2.301(.107)	2.277(.142)	2.398(112)	**t(39)=-2.796 p=.008**	**t(63)=-3.505 p<.001**	t(58)=.631 p=.530

Con= controls; NRe=non-responders; Re=responders.

Table S3. Subgroup comparisons for clusters significantly different between responders and non-responders. This table reports the results of the independent-sample t-tests *(two-tailed)* comparing morphometric measures between controls and (separately) non-responders and responders for clusters found significantly different between the two ADHD treatment response groups. Significant results are highlighted in bold.

As shown, only non-responders significantly differed from controls in these 10 clusters. Results are displayed in Fig.3.

Measure	Cluster	Region Labels	**Nre** mean (SD)	**Re** mean (SD)	**Con** mean (SD)	**Nre vs Con** t-test	**Re vs Con** t-test
Cortical Volume							
	1	banks superior temporal sulcus, inferior temporal gyrus, insula, middle temporal gyrus, postcentral gyrus, superior temporal gyrus, supramarginal gyrus, temporal pole, transverse temporal cortex	1.621 (.177)	1.830 (.162)	1.848 (.202)	**t(39)=-3.758 p<.001**	t(63)=-.388 p=.699
	2	precuneus , inferior and superior parietal cortices	1.323 (.145)	1.492 (.131)	1.492 (.130)	**t(39)=-3.904 p<.001**	t(63)=-.007 p=.994
	3	lateral orbital frontal cortex, rostral middle frontal gyrus	1.891 (.233)	2.130 (.202)	2.135 (.145)	**t(39)=-4.113 p<.001**	t(63)=-.108 p=.914
	4	insula, temporal pole, middle and superior temporal gyri	2.177 (.303)	2.499 (.283)	2.536 (.244)	**t(39)=-4.203 p<.001**	t(63)=-.524 p=.602
	5	fusiform gyrus, lateral occipital cortex, lingual gyrus, parahippocampal gyrus	2.005 (.224)	2.260 (.258)	2.251 (.182)	**t(39)=-3.871 p<.001**	t(63)=.152 p=.879
Surface Area							
	1	caudal middle frontal gyrus, insula, lateral orbital frontal cortex, pars triangularis, postcentral gyrus, precentral gyrus, rostral middle frontal gyrus	.794(.090)	.899 (.072)	.879 (.082)	**t(39)=-3.097 p=.002**	t(63)=1.013 p=.315
	2	banks superior temporal sulcus, insula, middle temporal gyrus, superior temporal gyrus, supramarginal gyrus, transverse temporal cortex	.581 (.062)	.656 (.058)	.633 (.077)	**t(39)=-2.326 p=.013**	t(63)=1.335 p=.187
	3	banks superior temporal sulcus, middle and superior temporal gyri	.771 (.075)	.879 (.086)	.853 (.081)	**t(39)=-3.302 p=.001**	t(63)=1.162 p= .250
	4	fusiform gyrus, lateral occipital cortex, lingual gyrus, pericalcarine cortex	.832 (.126)	.960 (.098)	.936 (.087)	**t(39)=-3.124 p=.002**	t(63)=.956 p= .343
	5	postcentral gyrus, superior parietal cortex	.524 (.074)	.596 (.072)	.585 (.087)	**t(39)=-2.333 p=.012**	t(63)=.539 p=.570

Con= controls; NRe=non-responders; Re=responders.

Table S4. Linear regressions. This table reports the results of the linear regressions testing whether morphometric differences between responders and non-responders were associated with improvement on inattentive and/or impulsive/hyperactive symptoms after two months of treatment. Significant results are highlighted in bold. Those that survived Bonferroni correction for multiple comparisons ($p \leq 0.005$) are displayed in Fig.4.

			Improvement BAARS-Ina			Improvement BAARS-Hyp/Imp		
Measure	Cluster	Region Labels	Equation	F(p-value)	R2	Equation	F(p-value)	R2
Cortical Volume Re-Nre								
	1	banks superior temporal sulcus, inferior temporal gyrus, insula, middle temporal gyrus, postcentral gyrus, superior temporal gyrus, supramarginal gyrus, temporal pole, transverse temporal cortex	SI=-3.321+4.443 CV	**6.414(.014)**	0.1	SI=-1.174+2.219 CV	1.064(.306)	0.018
	2	precuneus , inferior and superior parietal cortices	SI=-3.533+5.595 CV	**6.737(.012)**	0.104	SI=-4.962+5.350 CV	**4.300(.043)**	0.069
	3	lateral orbital frontal cortex, rostral middle frontal gyrus	SI=-3.917+4.104 CV	**8.713(.005)**	0.131	SI=-4.260+3.405 CV	**4.045(.049)**	0.065
	4	insula, temporal pole, middle and superior temoral gyri	SI=-1.233+2.400 CV	**5.253(.026)**	0.83	SI=.403+.977 CV	.585(.448)	0.01
	5	fusiform gyrus, lateral occipital cortex, lingual gyrus, parahippocampal gyrus	SI=-.124+2.132 CV	2.843(.097)	0.47	SI=-1.493+1.943 CV	1.679(.200)	0.028
Surface Area Re-Nre								
	1	caudal middle frontal gyrus, insula, lateral orbital frontal cortex, pars triangularis, postcentral gyrus, precentral gyrus, rostral middle frontal gyrus	SI=-5.910+12.033 SA	**11.628(.001)**	0.167	SI=-5.020+8.953 SA	**4.170(.046)**	0.067
	2	banks superior temporal sulcus, insula, middle temporal gyrus, superior temporal gyrus, supramarginal gyrus, transverse temporal cortex	SI=-2.296+10.773 SA	**4.723(.034)**	0.075	SI=-3.435+9.757 SA	2.721(.104)	0.045
	3	banks superior temporal sulcus, middle and superior temporal gyri	SI=-4.926+11.167 SA	**11.017(.002)**	0.16	SI=-6.377+10.775 SA	**7.009(.010)**	0.108
	4	fusiform gyrus, lateral occipital cortex, lingual gyrus, pericalcarine cortex	SI=-2.933+8.098 SA	**8.980(.004)**	0.134	SI=-4.986+8.391 SA	**6.763(.012)**	0.104
	5	postcentral gyrus, superior parietal cortex	SI=-2.377+12.011 SA	**8.316(.006)**	0.125	SI=-1.277+7 SA	1.849(.179)	0.031
Cortical Volume ADHD-Con								
	3	fusiform gyrus, inferior and middle temporal gyri	SI=-.883+2.139 CV	3.589(.063)	0.058	SI=3.517-.303 CV	.049(.825)	0.001

Con= controls; NRe=non-responders; Re=responders.

Imaging-transcriptomic analyses (virtual histology)

We examined the genomic associates of the observed morphometric differences between ADHD and neurotypical controls, and between ADHD responders and non-responders to MPH treatment. As shown in Figures S1 and S2, ADHD vs controls differences in CT were enriched for genes associated with the transport of NA (CT Odds ratio [OR]=13.012, pFDR=.028); and for genes expressed by astrocytes during neurodevelopment (CT OR=2.93, pFDR=.014). We observed a trend for interneurons (CT OR=10.262, pFDR=.06). Morphometric differences between responders and non-responders did not show significant enrichment for the identified genes. Full results are reported in Table S5-6.

<u>Enrichment analysis for genes implicated in neurotransmission</u>

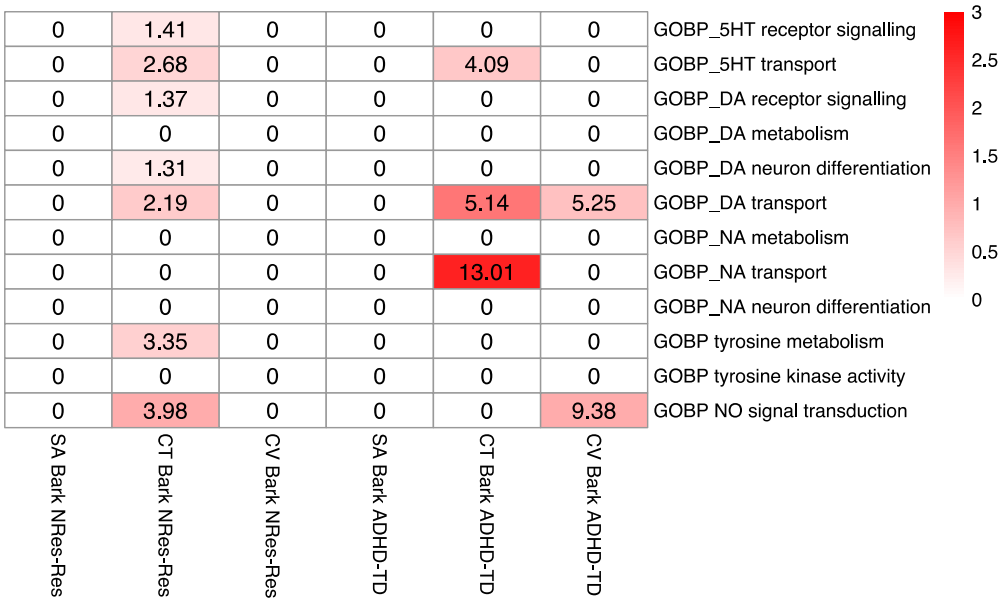

FIGURE S1. Enrichment analysis for genes implicated in neurotransmission. This shows the results of the enrichment analysis for cortical morphometric measures (rows) by genes implicated in neurotransmission pathways and potentially associated with MPH treatment response (columns) (see also Table S5). The tested genes were based on gene-set annotations listed in the human gene ontology database (http://www.gsea-msigdb.org/)[3].Tile colors indicate false discovery rate (FDR) p-values. Tile labels indicate enrichment odds ratios.

Table S5. Enrichment analysis for genes implicated in neurotransmission. This table shows odds ratio and pFDR of the enrichment analysis for cortical morphometric measures by genes implicated in neurotransmission pathways and potentially associated with MPH treatment response. The tested genes were based on gene-set annotations listed in the human gene ontology database (http://www.gsea-msigdb.org/)[3]. Significant results are highlighted in bold.

Neurotransmission	Odds ratio						*pFDR*					
	SA NRes-Res	CT NRes-Res	CV NRes-Res	SA ADHD-Con	CT ADHD-Con	CV ADHD-Con	SA NRes-Res	CT NRes-Res	CV NRes-Res	SA ADHD-Con	CT ADHD-Con	CV ADHD-Con
5HT receptor signalling	0	1.411	0	0	0	0	1	0.938	1	1	1	1
5HT transport	0	2.683	0	0	4.088	0	1	0.938	1	1	0.914	1
DA receptor signalling	0	1.375	0	0	0	0	1	0.938	1	1	1	1
DA metabolism	0	0.000	0	0	0	0	1	1.000	1	1	1	1
DA neuron differentiation	0	1.307	0	0	0	0	1	0.938	1	1	1	1
DA transport	0	2.193	0	0	5.144	5.250	1	0.938	1	1	0.148	1
NA metabolism	0	0.000	0	0	0	0	1	1.000	1	1	1	1
NA transport	0	0.000	0	0	**13.013**	0	1	1.000	1	1	**0.028**	1
NA neuron differentiation	0	0.000	0	0	0	0	1	1.000	1	1	1	1
Tyrosine metabolism	0	3.354	0	0	0	0	1	0.938	1	1	1	1
Tyrosine kinase activity	0	0.000	0	0	0	0	1	1.000	1	1	1	1
NO signal transduction	0	3.984	0	0	0	9.385	1	0.938	1	1	1	1

Enrichment analysis for genes expressed by brain cell type during development

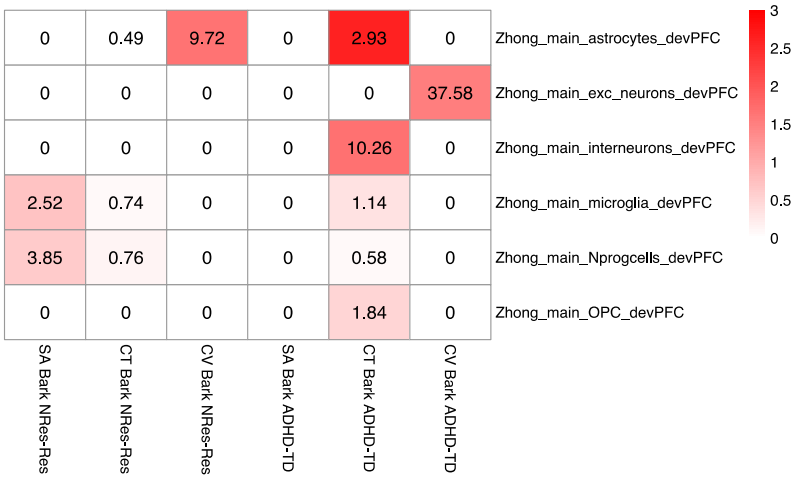

FIGURE S2. Enrichment analysis for genes expressed by cell type during development. This shows the results of the enrichment analysis for cortical morphometric measures (rows) by genes implicated in cell type during development (see also table S6). The cell type-specific gene lists were based on prior work [4] and are available at (http://www.gsea-msigdb.org/). Tile colors indicate false discovery rate (FDR) p-values. Tile labels indicate enrichment odds ratios.

Table S6. Enrichment analysis for genes expressed by cell type during development. This table shows odds ratio and pFDR of the enrichment analysis for cortical morphometric measures by genes implicated in cell type development. The cell type-specific gene lists were based on prior work [4] and are available at (http://www.gsea-msigdb.org/). Significant results are highlighted in bold. Trend results are highlighted in italics.

Cell types	Odds ratio						*pFDR*					
	SA NRes-Res	CT NRes-Res	CV NRes-Res	SA ADHD-Con	CT ADHD-Con	CV ADHD-Con	SA NRes-Res	CT NRes-Res	CV NRes-Res	SA ADHD-Con	CT ADHD-Con	CV ADHD-Con
Astrocytes	0	0.494	9.717	0	**2.930**	0	1	1	0.135	1	**0.014**	1
Excitatory neurons	0	0.000	0	0	0.000	37.577	1	1	1	1	1.000	0.182
Interneurons	0	0.000	0	0	10.262	0	1	1	1	1	*0.060*	1
Microglia	2.523	0.740	0	0	1.135	0	0.704	1	1	1	0.675	1
Neuroanl progenitor cells	3.853	0.764	0	0	0.576	0	0.704	1	1	1	0.993	1
Oligodendrocyte progenitor cells	0	0	0	0	1.838	0	1	1	1	1	0.615	1

ABBREVIATIONS

AHBA=Allen Human Brain Atlas; BAARS-IV= Barkley Adult ADHD Rating Scale-IV; BAARS-INA= Barkley Adult ADHD Rating Scale-IV -inattentive symptoms; BAARS-HI= Barkley Adult ADHD Rating Scale-IV- hyperactive/impulsive symptoms; Con=controls; CT=cortical thickness; DA=dopamine; FDR= false discovery rate; 5-HT=5-hydroxytryptamine; ICV= intracranial volume; IQ= intelligence quotient; MPH= methylphenidate; NA=noradrenaline; NO= nitric oxide; NRe=non-responders; OR= odds ratio; Re=responders; SA= surface area.

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