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Lower fractional anisotropy of cortico-thalamic tract and increased response time variability in adult patients with ADHD

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– Supplementary Information –

Given what has been reported before[1, 2], we also employed an exploratory voxel-wise whole-brain analysis in the ADHD group, that is, leaving aside whether FA-values significantly differ between patients and controls. A regression analysis, assessed at an uncorrected voxel-height threshold $p < 0.005$, and $p < 0.05$ (family-wise error rate (FWE)-corrected) at the cluster level, revealed one significant cluster along the left corpus callosum, where tau was negatively related with FA-values (peak voxel: -18, 14, 28; z-value: 3.62, $p < 0.001$; cluster size: 206 voxels; $p = 0.031$, FWE-corrected).

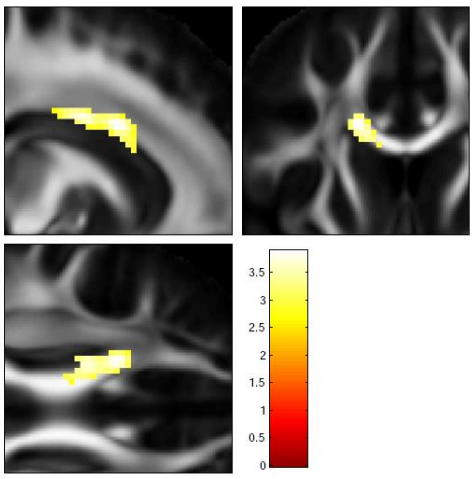

Figure S1. Within-group (ADHD) significant negative relationship between FA-values of the left callosal tract and tau, rendered on sagittal, coronal and transversal slices of the average FA template from the

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Illinois Institute of Technology Human Brain Atlas[3]. The color bar shows the color-coded height of t-values obtained from the single tailed t-contrast computed with a simple regression model. Significance levels: $p < 0.005$, uncorrected at the voxel-level, and $p < 0.05$, FWE-corrected at the cluster level.

To remain consistent with the analysis in the main paper, FA-values from this cluster were averaged across voxels per each ADHD patient and then subjected to a multiple regression analysis. Results are summarized in Table S1 and Figure S1. Only averaged FA-values significantly predicted response time variability (tau) with an effect size (Cohen´s d) of 1.05. None of the other predictors was significant.

Table S1. Summary of the multiple regression analysis with tau as the dependent variable.

Predictor variables	beta	SEM	t	p	-95% CI	+95% CI	Partial eta²	Cohen's d
Averaged FA	-0.493	0.137	-3.60	0.0008	-0.770	-0.218	0.216080	1.050
Age	-0.002	0.137	-0.01	0.990	-0.278	0.274	0.000004	0.004
Sex	-0.047	0.139	-0.34	0.736	-0.328	0.233	0.002439	0.099
Years of School	-0.013	0.135	-0.10	0.925	-0.285	0.260	0.000193	0.028
Estimated IQ	0.099	0.129	0.77	0.447	-0.160	0.358	0.012338	0.224

CI: confidence interval; SEM: standard error of the mean.

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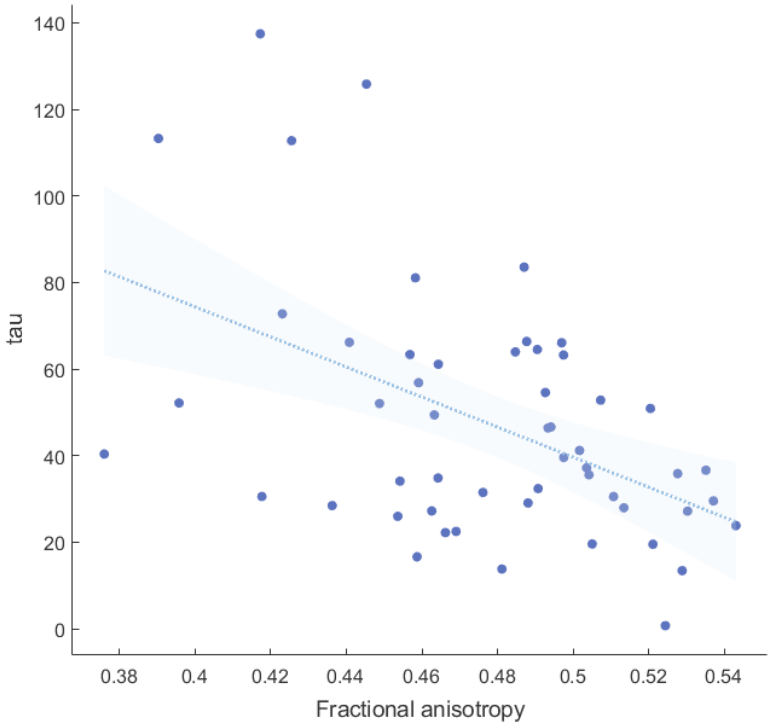

Figure S2. Significant negative relationship between averaged FA-values of the left callosal tract and response time variability (tau). The line represents the regression line, the shaded area represents the 95% confidence interval. The plot was created using the Gramm toolbox[4].

Again, we used data from the healthy comparisons to control whether this group would also show a negative relationship between FA-values and tau in the same cluster significant in the ADHD group. However, already in the exploratory whole-brain regression model none of the in-mask voxels was significant ($p > 0.1$) for this group. We also tested whether FA-values of this specific cluster would show between-group differences in FA-values, which was not the case even at a threshold as low as $p = 0.2$, that is, averaged FA-values of this cluster were almost identical between both groups. Lastly, a whole brain regression analysis in the control group did not reveal any association between tau and FA when applying the same levels of significance as in the ADHD group ($p < 0.005$, uncorrected at the voxel-level, and $p < 0.05$, FWE-corrected at the cluster level).

Replication analysis using FSL's Tract-based Spatial Statistics (TBSS)

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Motivated by the reviewing procedure, this section contains results from a replication analysis for all results reported in the main article and the supplemental analyses reported above, group-analyzing FA-values with FMRIB Software Library (FSL)'s standard TBSS pipeline[5]. For this, all the necessary scripts (tbss_1_preproc, tbss_2_reg, tbss_3_postreg, tbss_4_prestats) as delivered with the release version FSL 6.0.2 were executed as is, with the following exceptions for reasons of comparability with the atlas-based analysis reported in the main article (see Methods section): for the script tbss_3_postreg the flag –T has been set in order to use the FMRIB58_FA mean FA image as reference, and the threshold for FA-values has been set to 0.1. As in the main paper, group comparisons and regression analyses were computed using corresponding routines from the software package Statistical Parametric Mapping (SPM12 r7487, Wellcome Department of Cognitive Neurology, London, UK). Where appropriate, Threshold-free Cluster Enhancement (TFCE) results were computed by means of the TFCE toolbox (r223; 08-12-2021) from Christian Gaser's group, Jena, Germany (http://www.neuro.uni-jena.de/tfce/) with 5000 iterations, and 2D optimization for TBSS DTI data set switched on (internal TFCE parameters H = 2 and E = 1).

First, the central analysis in the manuscript was repeated to infer group differences between healthy controls and patients with ADHD, and to test whether decreased FA-values in the ADHD group would relate to increased values of tau. Group-differences (Figure S3 A and B) in FA-values of the cortico-thalamic tract could be replicated with the data from the TBSS pipeline, although locations of between-group differences from both data-processing streams did not exactly match, given the major differences between both analysis pipelines: normalization, spatial resolution, smoothing, and means of inference (i.e., voxel-wise and cluster-based thresholding in smoothed 3D-data versus TFCE-based inference in unsmoothed 2D data).

To test for the relationship between tau and significantly decreased FA-values in the ADHD group, the multiple regression analyses were repeated for TBSS data, this time however, separately for FA-values

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from left and right cortico-thalamic tract, because averaged FA-values from both tracts did less highly correlate across ADHD patients ($r = 0.64$; $p < 0.001$; correlation coefficient as reported in the main article was $r = 0.81$). For the left cortico-thalamic tract, the relationship was not significant (beta = -0.15; $t = -1.00$; $p = 0.318$), while it preserved its sign and significance for the right cortico-thalamic tract; see Table S2 below, and the corresponding scatter plot in Figure S3 C. To control for the ADHD specificity of this result, the same multiple regression analysis was repeated in healthy controls with no significant results as reported in the main article (left cortico-thalamic tract: beta = 0.03; $t = 0.21$; $p = 0.836$; right cortico-thalamic tract: beta = 0.14; $t = 0.92$ $p = 0.360$).

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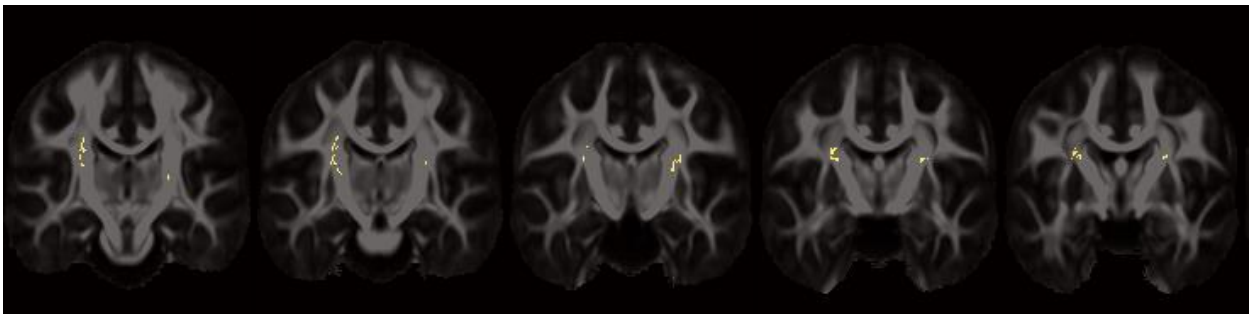
A)



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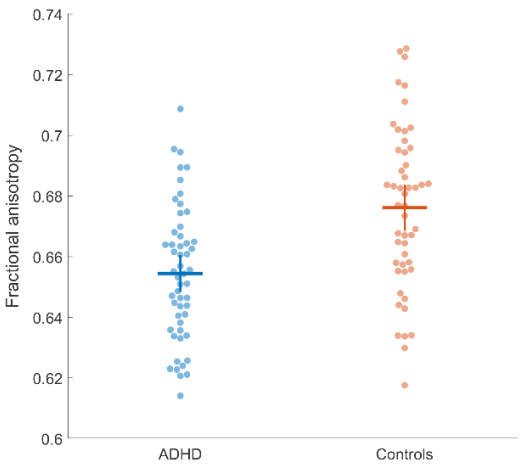

C)

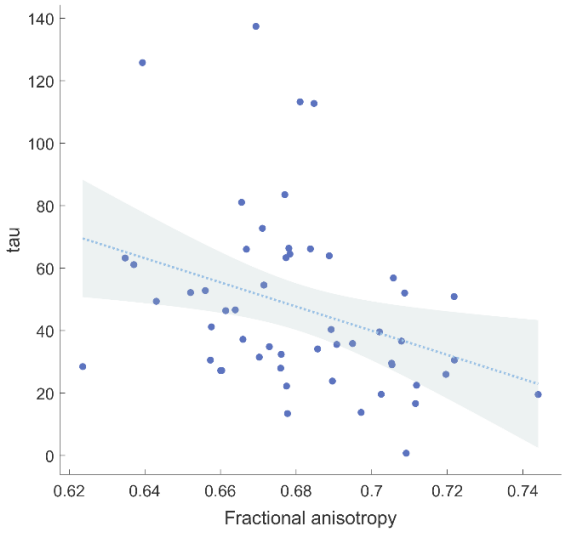

Figure S3. A) TBSS-based between-group comparison of fractional anisotropy in the cortico-thalamic tract; unsmoothed data with 1 mm isotropic spatial resolution; TFCE-based inference of significant between-group differences in FA-values in the single-tailed direction of controls minus ADHD patients (level of inference: $p < 0.025$, FWE-corrected and a cluster extent threshold of 100 contiguously significant voxels to replicate results from the atlas-based approach reported in the manuscript). **B)** Beeswarm plots, means and 95% confidence intervals of FA-values for both groups, averaged across all significant voxels from the analysis above. **C)** Scatter plot of average FA-values from right cortico-thalamic tact in the group of 53 ADHD patients to predict individual variations in tau; results from the corresponding multiple regression analysis for this relationship is tabulated below (Table S2).

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Table S2. Summary of the multiple regression analysis with tau as the dependent variable and averaged FA-values from the right cortico-thalamic tract where ADHD patients showed significantly lower FA-values relative to healthy controls as the predictor variable of interest.

Predictor variables	beta	SEM	t	p	-95% CI	+95% CI	Partial eta^2	Cohen's d
Averaged FA	-0.348	0.153	-2.27	0.028	-0.656	-0.040	0.099007	0.663
Age	0.085	0.143	0.59	0.556	-0.203	0.372	0.007411	0.173
Sex	-0.081	0.156	-0.52	0.605	-0.395	0.233	0.005742	0.152
Years of School	-0.021	0.145	-0.14	0.888	-0.313	0.273	0.000427	0.041
Estimated IQ	-0.007	0.142	-0.05	0.963	-0.293	0.280	0.000045	0.013

CI: confidence interval; SEM: standard error of the mean.

Next, using the TBSS data we also repeated the exploratory analysis presented above at the beginning of this Supplementary Information file. That is, this analysis tested for a relationship between tau and FA-values in ADHD patients, leaving aside whether these values significantly differ between patients and healthy controls. At the same level of significance as reported above, ADHD patients revealed a significant negative relationship between tau and FA-values of the left callosal tract (adjunct to the left cingulum bundle) as already reported above (peak voxel: -19, 19, 30; z-value = 4.17; $p < 0.001$; cluster size: 82 voxels; $p = 0.004$, FWE-corrected; Figure S4), while the spatial extent of this effect was smaller given that unsmoothed data were used at an isotropic spatial resolution of 1 mm.

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A) **B)**

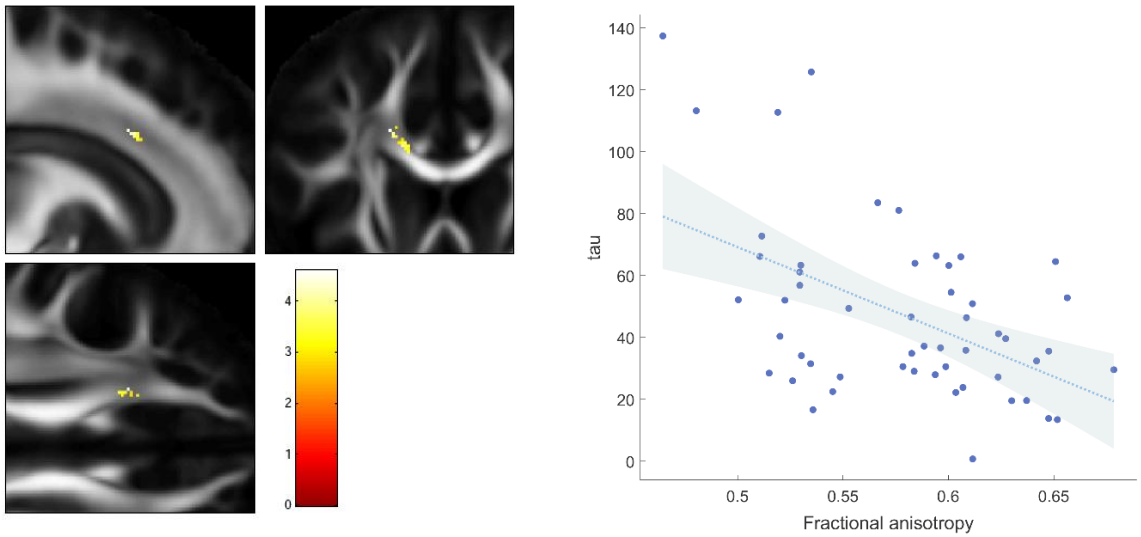

Figure S4. A) Within-group (ADHD) significant negative relationship between FA-values of the left callosal tract (adjunct to the cingulum bundle) and tau, rendered on sagittal, coronal and transversal slices of the average FA template from the Illinois Institute of Technology Human Brain Atlas[3]. **B)** The scatter plot on the right side graphically depicts the relationship between averaged FA-values and tau. Results from the corresponding multiple regression analysis are tabulated below (Table S3).

Table S3. Summary of the multiple regression analysis with tau as the dependent variable and averaged FA-values from the left callosal tract/cingulum bundle of ADHD patients as the predictor variable of interest.

Predictor variables	beta	SEM	t	p	-95% CI	+95% CI	Partial eta²	Cohen's d
Averaged FA	-0.498	0.129	-3.86	< 0.001	-0.76	-0.24	0.240717	1.126
Age	0.073	0.130	0.56	0.577	-0.19	0.33	0.006659	0.164
Sex	-0.021	0.136	-0.15	0.878	-0.29	0.25	0.000504	0.045
Years of School	-0.093	0.133	-0.70	0.490	-0.36	0.18	0.010216	0.203
Estimated IQ	0.044	0.127	0.35	0.729	-0.21	0.30	0.002569	0.102

CI: confidence interval; SEM: standard error of the mean.

Finally, repetition of the exploratory control analysis with TBSS data from healthy controls did not show any voxels bearing a significant relationship between FA-values and tau at the whole-brain level when

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applying the same levels of significance as in the ADHD group, nor did this group reveal any association ($p > 0.1$) for in-mask voxels derived from the above regression analysis in ADHD patients. TBSS-derived FA data between both groups did also not differ for this location even at a threshold as low as $p = 0.2$, that is, FA-values of this cluster were almost identical between both groups (as already reported above).

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