PNAS

Supporting Information for

Hemispheric asymmetry in cortical thinning reflects intrinsic organization of the neurotransmitter systems and homotopic functional connectivity

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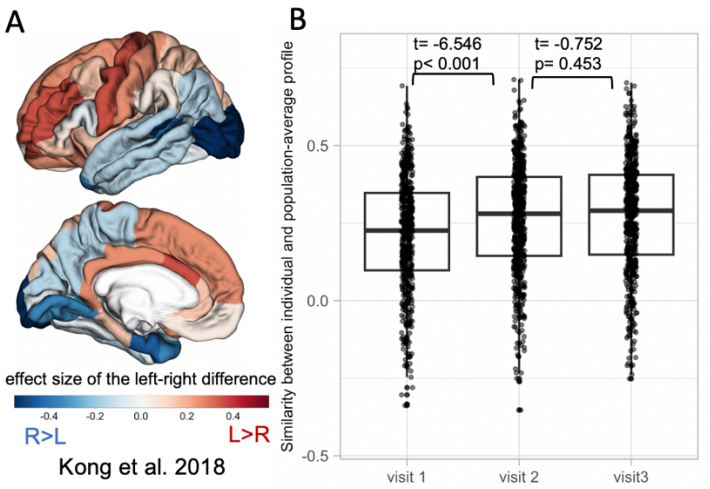

Fig. S1. Similarity of individual-ENIGMA thickness asymmetry profile. (A) The profile of left-right differences in thickness from ENIGAM study (1). Positive values (in red) indicate the left hemisphere is thicker than the right. (B) From adolescence to adulthood, individuals' thickness asymmetry profiles became more similar to this population-average profile of thickness asymmetry.

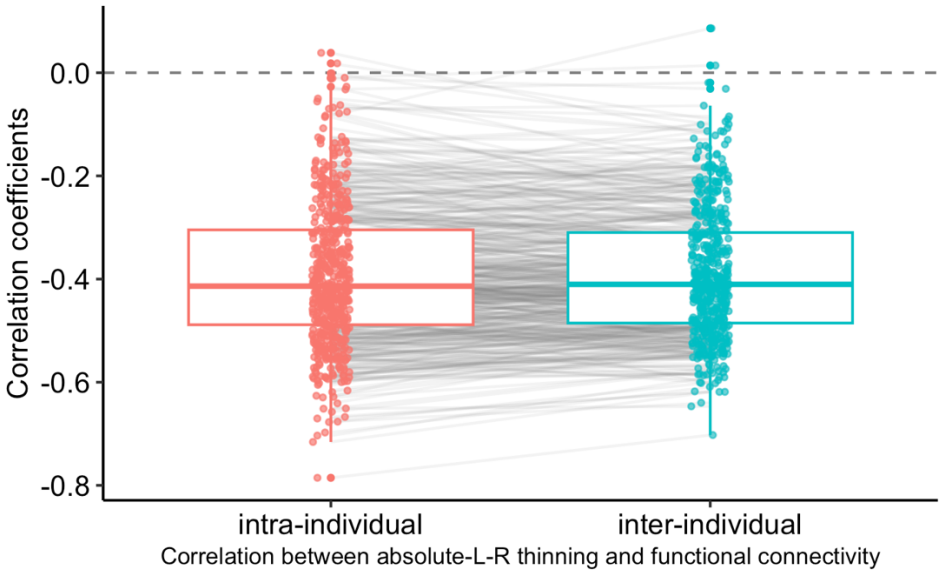

Fig. S2. Correlations between individuals' absolute-L-R differences in thinning and their own homotopic functional connectivity (intra-individual correlations), and the others' homotopic functional connectivity (inter-individual correlations).

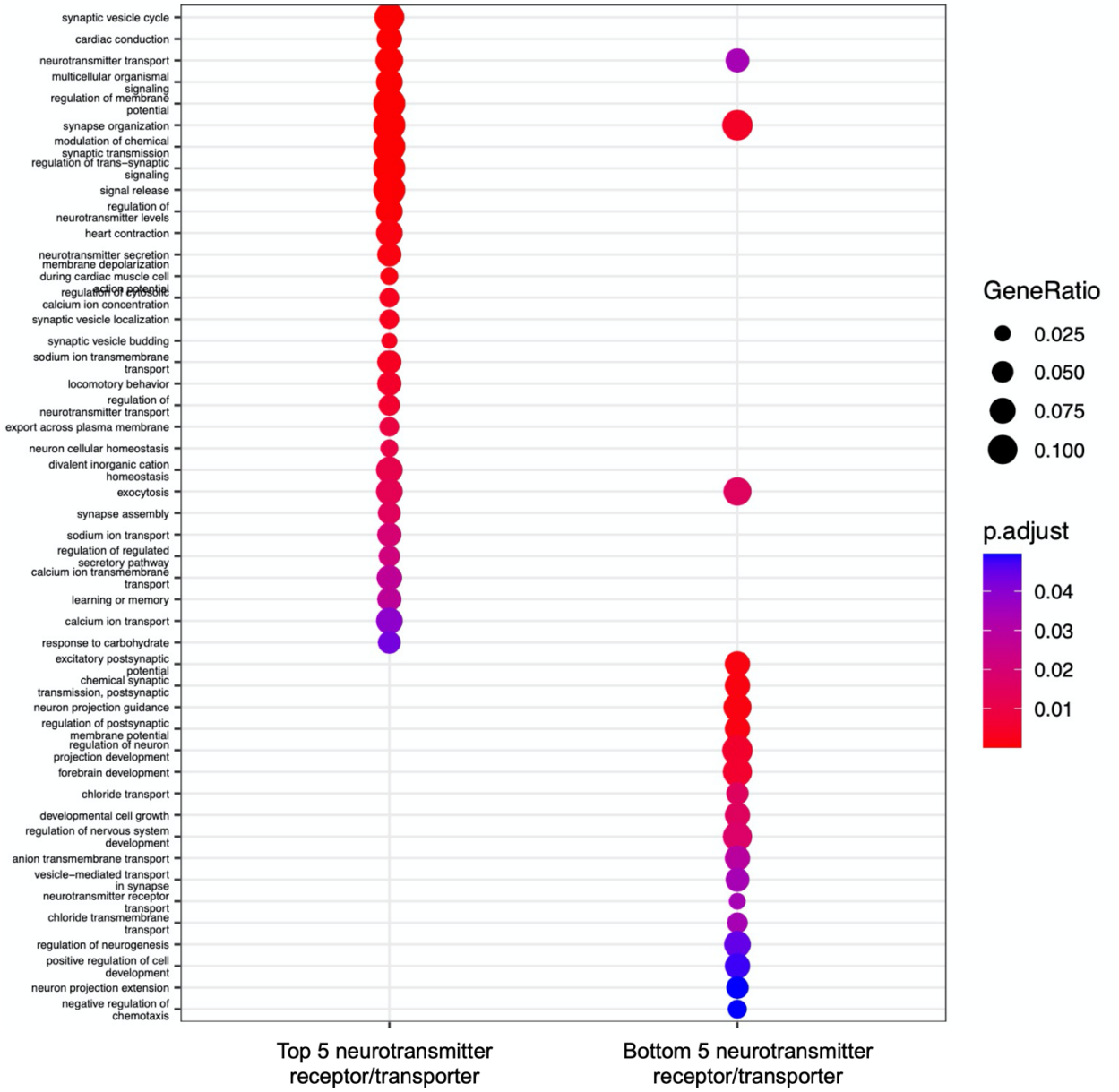

Fig. S3. GO term analysis with co-expression genes of neurotransmitter receptor and transporter genes. This analysis was done to explore possible biological functions of neurotransmitter receptor/transporters that had the strongest relationship with the hemispheric differences in cortical thinning. The seed genes were selected as the genes of the top 5 and bottom 5 neurotransmitter receptor/transporters with the strongest relationship with the hemispheric differences in cortical thinning. Each seed gene was co-expressed against all other genes within a harmonized dataset of gene expression in human cerebral cortex (534 donors) using a linear mixed effects model with age, hemisphere, and sex as fixed effects, and region and donor id as random effects (2). The genes of the top 5 neurotransmitter receptor/transporters, or the seed genes of the co-expression analyses, are: GRIN1 (NMDA), GRIN2A (NMDA), GRIN2B (NMDA), SLC6A3 (DAT), OPRM1 (MOR), SLC18A3 (VAChT), HTR1B (HTRB). The genes of the bottom 5 neurotransmitter receptor/transporters are: HTR2A (5HT2a), SLC6A4 (5HTT), DRD2 (D2), CNR1 (CB1), CHRNA4 (a4b2), CHRNB2 (a4b2). The top 10 of positively co-expressed genes for each seed gene were used to construct the top and bottom neurotransmitter receptor/transporters co-expressed panels. Gene-ontology (GO) enrichment analysis was conducted using the ‘clusterProfiler’ R package (3). Only GO terms

with a minimum of 10, and maximum of 500 genes were tested, and redundant terms were removed with a similarity cut-off of 0.7 (default parameters).

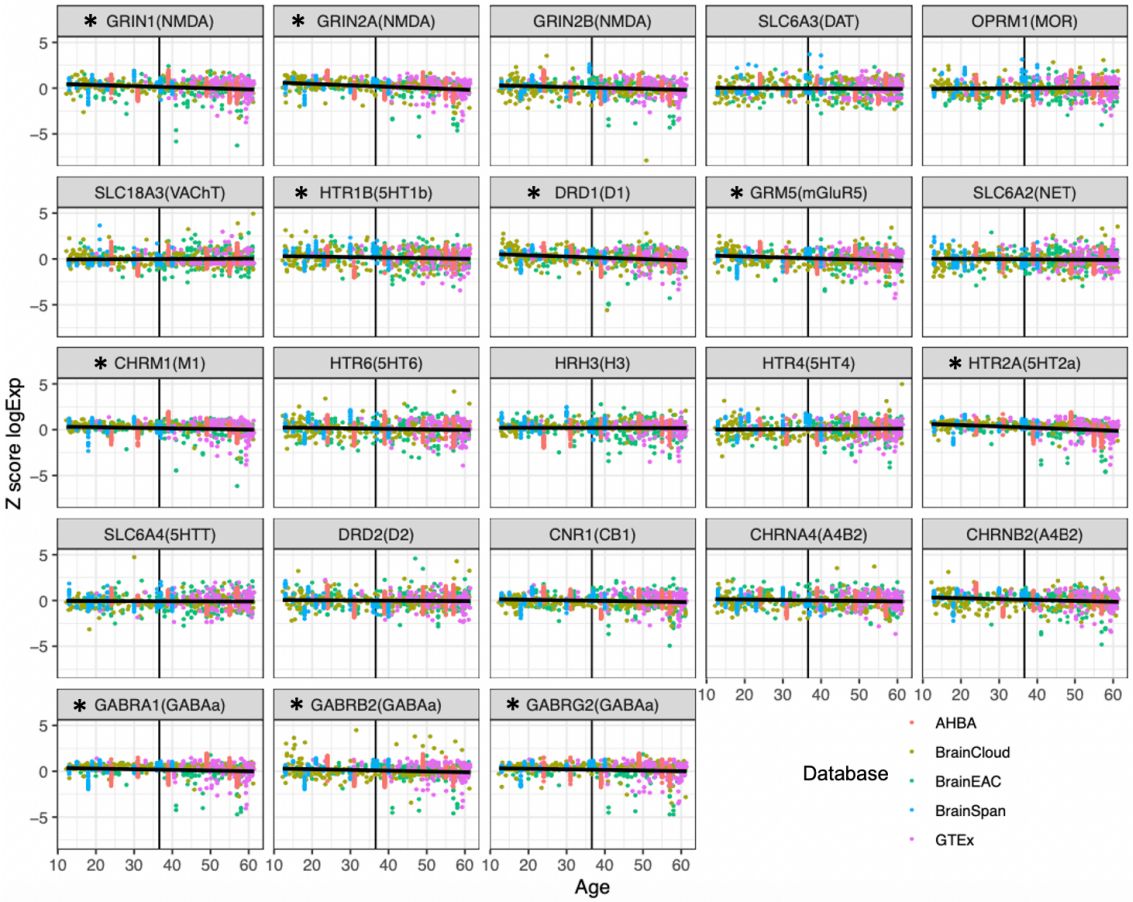

Fig. S4. Age-related variation in expression of neurotransmitter receptor and transporter genes in cerebral cortex. This harmonized dataset included five human post-mortem gene expression databases: Allen Human Brain Atlas, BrainCloud, the Brain eQTL Almanac, the Genotype- Tissue Expression Project, and BrainSpan. The age range of interest, 11.6 - 61.6 years, was determined by (1) age range of cortical thinning in this study: 14 – 22 years; (2) mean sample-size weighted age of participants in PET studies 36.6 years ±25 years. The mean age is indicated by the vertical line in each plot. The raw expression values of each gene were log transformed and normalized (by z scoring) within each sampled region and within each database. "*" indicates there was significant association between age and the gene expression of the gene. The age-expression correlations were estimated with linear mixed effect models which included age, sex and region as fixed effects, and donor ID as random effect. The analyses were done with R packages "lme4", "lmerTest" and "MuMIn".

Table S1. Cortical thinning in each region and hemispheric differences in thinning

regions	lh.thinning	rh.thinning	hemispheric differences in thinning	t.value (L-R differences)	p.value (L-R differences)	p.fdr (L-R differences)
bankssts	0.115	0.124	-0.009	-1.450	1.48E-01	2.09E-01
caudalanteriorcingulate	0.103	0.104	-0.002	-0.182	8.56E-01	8.56E-01
caudalmiddlefrontal	0.111	0.137	-0.026	-4.620	4.82E-06	1.49E-05
cuneus	0.110	0.094	0.016	3.938	9.30E-05	2.11E-04
entorhinal	-0.093	-0.088	-0.005	-0.305	7.61E-01	7.84E-01
fusiform	0.029	0.031	-0.002	-0.431	6.67E-01	7.08E-01
inferiorparietal	0.170	0.150	0.020	4.444	1.08E-05	3.05E-05
inferiortemporal	-0.054	0.032	-0.086	-11.838	7.41E-29	1.26E-27
isthmuscingulate	0.087	0.080	0.007	1.239	2.16E-01	2.82E-01
lateraloccipital	0.104	0.106	-0.002	-0.449	6.53E-01	7.08E-01
lateralorbitofrontal	0.063	0.110	-0.047	-7.458	3.59E-13	2.04E-12
lingual	0.085	0.088	-0.003	-0.835	4.04E-01	4.90E-01
medialorbitofrontal	0.100	0.116	-0.016	-2.387	1.73E-02	3.28E-02
middletemporal	-0.014	0.092	-0.106	-16.600	3.72E-50	1.27E-48
parahippocampal	0.004	0.025	-0.021	-2.661	8.02E-03	1.60E-02
paracentral	0.130	0.124	0.006	1.283	2.00E-01	2.72E-01
parsopercularis	0.086	0.124	-0.038	-7.160	2.70E-12	1.31E-11
parsorbitalis	0.084	0.135	-0.050	-5.579	3.86E-08	1.46E-07
parstriangularis	0.087	0.153	-0.067	-10.943	2.84E-25	3.22E-24
pericalcarine	0.047	0.056	-0.009	-2.164	3.09E-02	5.53E-02
postcentral	0.097	0.104	-0.007	-1.903	5.75E-02	9.31E-02
posteriorcingulate	0.112	0.115	-0.003	-0.539	5.90E-01	6.69E-01
precentral	0.054	0.081	-0.027	-6.171	1.35E-09	5.72E-09
precuneus	0.142	0.126	0.015	4.144	3.96E-05	1.04E-04
rostralanteriorcingulate	0.074	0.097	-0.023	-2.115	3.49E-02	5.94E-02
rostralmiddlefrontal	0.096	0.156	-0.060	-10.504	1.41E-23	1.20E-22
superiorfrontal	0.156	0.177	-0.021	-5.227	2.48E-07	8.43E-07
superiorparietal	0.144	0.138	0.006	1.852	6.46E-02	9.98E-02
superiortemporal	0.039	0.085	-0.045	-9.678	1.65E-20	1.12E-19
supramarginal	0.138	0.145	-0.007	-1.453	1.47E-01	2.09E-01
frontalpole	0.107	0.151	-0.043	-3.237	1.28E-03	2.73E-03
temporalpole	-0.101	-0.120	0.019	1.107	2.69E-01	3.38E-01
transversetemporal	0.098	0.128	-0.030	-4.026	6.51E-05	1.58E-04
insula	0.088	0.082	0.006	0.810	4.18E-01	4.90E-01

Table S2. Age-related variation in expression of neurotransmitter receptor and transporter genes in cerebral cortex using linear mixed effect models. The genes are listed in the order of the p values with FDR correction (P.fdr).

Receptor/transporter	Gene symbol	Beta	r2	P.value	P.fdr	Correlated with hemispheric differences in thinning
NMDA	***GRIN2A***	**-0.0166**	**0.065**	**1.29E-07**	**2.97E-06**	**Yes**
5HT2a	***HTR2A***	**-0.0143**	**0.057**	**6.12E-07**	**7.03E-06**	**No**
GABAa	***GABRA1***	**-0.0128**	**0.045**	**9.81E-06**	**7.52E-05**	**No**
D1	***DRD1***	**-0.0134**	**0.04**	**3.21E-05**	**1.85E-04**	**Yes**
mGluR5	***GRM5***	**-0.0132**	**0.038**	**4.39E-05**	**2.02E-04**	**Yes**
M1	***CHRM1***	**-0.0101**	**0.03**	**2.47E-04**	**9.47E-04**	**No**
5HT1b	***HTR1B***	**-0.0108**	**0.025**	**6.97E-04**	**2.29E-03**	**Yes**
NMDA	***GRIN1***	**-0.0093**	**0.022**	**1.69E-03**	**4.85E-03**	**Yes**
GABAa	***GABRG2***	**-0.0091**	**0.02**	**3.46E-03**	**8.84E-03**	**No**
GABAa	***GABRB2***	**-0.0092**	**0.015**	**1.13E-02**	**2.60E-02**	**No**
H3	*HRH3*	-0.0058	0.01	2.86E-02	5.99E-02	No
NMDA	*GRIN2B*	-0.0075	0.011	3.14E-02	6.03E-02	Yes
A4B2	*CHRNB2*	-0.0059	0.008	7.22E-02	1.28E-01	No
5HTT	*SLC6A4*	0.0043	0.006	9.53E-02	1.57E-01	No
5HT6	*HTR6*	-0.0047	0.005	1.19E-01	1.83E-01	No
CB1	*CNR1*	-0.0042	0.005	1.34E-01	1.92E-01	No
A4B2	*CHRNA4*	-0.0041	0.004	1.61E-01	2.18E-01	No
D2	*DRD2*	-0.0037	0.003	1.96E-01	2.51E-01	No
MOR	*OPRM1*	-0.0014	0.001	6.30E-01	7.62E-01	Yes
DAT	*SLC6A3*	0.0012	0	6.73E-01	7.74E-01	Yes
5HT4	*HTR4*	-0.0012	0	7.18E-01	7.87E-01	No
VAChT	*SLC18A3*	-0.0004	0	8.70E-01	9.10E-01	Yes
NET	*SLC6A2*	0.0001	0	9.70E-01	9.70E-01	Yes

Bold fonts indicate significant age-expression correlations.

Dataset S1 (separate file). Type or paste legend here. Hemispheric differences in the density of each of 19 neurotransmitter receptors and transporters. “Receptor differences” was calculated by summing up the absolute hemispheric differences of the 19 receptors/transporters.

SI References

1. X.-Z. Kong, *et al.*, Mapping cortical brain asymmetry in 17,141 healthy individuals worldwide via the ENIGMA Consortium. *Proceedings of the National Academy of Sciences* **115**, E5154–E5163 (2018).

2. N. Parker, *et al.*, Assessment of Neurobiological Mechanisms of Cortical Thinning During Childhood and Adolescence and Their Implications for Psychiatric Disorders. *JAMA Psychiatry* **77**, 1127–1136 (2020).

3. T. Wu, *et al.*, clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)* **2**, 100141 (2021).

IMAGEN Consortium

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