Resective surgery prevents progressive cortical thinning in temporal lobe epilepsy: evidence for neuroprotection.

-- ONLINE SUPPLEMENT --

1. MRI acquisition protocol in epilepsy cohort

MRI data were acquired between August 2004 and March 2013 on the same 3T MRI GE Signa HDx scanner (GE, Milwaukee, WI, USA), with the same MRI image sequence used for analysis: coronal T1W 3D inversion-recovery fast spoiled gradient echo (IR-FSPGR) with repetition time / echo time / inversion time = 8.1 / 3.1 / 450 ms; field-of-view 187x240x240 mm; matrix 170x256x256, voxel dimensions 0.9x0.9x1.1mm.

2. Description of healthy volunteer cohorts

Healthy volunteer data in public repositories is mostly cross-sectional. A small number of longitudinal healthy volunteer datasets are available but they were mostly acquired as comparison for disease cohort of autism or dementia - i.e. the included volunteers are young (<20 years-old) or old (>70 years-old). We detected three publicly available cohorts of healthy volunteers aged between 20 to 70 years, having two 3T T1-weighted MRI scans on the same scanner at least 6 months apart.

Supplemental Table 1: *Healthy volunteer demographic by cohort*

	NMorphCH (n = 24)	PPMI (n = 48)	SLIM (n = 53)
Sex			
Female	10 (42%)	19 (40%)	47 (89%)
Male	14 (58%)	29 (60%)	6 (11%)
Age at baseline scan *(years)*	32 ± 9	56 ± 9	21 ± 1
Interval between scans *(years)*	1.7 ± 0.4	1.2 ± 0.4	2.1 ± 0.6

2.1 Neuromorphometry by Computer Algorithm Chicago (NMorphCH)

Number of volunteers: 24
Reference for Dataset: http://nunda.northwestern.edu/nunda/data/projects/NMorphCH
Obtained through: ShizConnect
Reference for ShizConnect: *Kogan A, Alpert K, Ambite JL, Marcus DS, Wang L. Northwestern University schizophrenia data sharing for SchizConnect: A longitudinal dataset for large-scale integration. Neuroimage 2016; 124: 1196–201.*
MR-acquisition: 3T Siemens TrioTim MRI scanner (Siemens Medical, Erlangen, Germany). A magnetization-prepared rapid gradient echo (MPRAGE) sequence was used to acquire high-resolution T1-weighted anatomical images (repetition time=2400 ms, echo time=3.16 ms, flip=8°, 256 x 256 matrix, 176 slices, slice thickness=1.0 mm, voxel size=1x1x1mm^3)

2.2 Parkinson Progression Marker Initiative (PPMI)

Number of volunteers: 48

Reference for Dataset: *Parkinson Progression Marker Initiative. The Parkinson Progression Marker Initiative (PPMI). Progress in Neurobiology 2011; 95: 629-35.*
MR-acquisition: Scans used for this longitudinal cohort were acquired on 3T Siemens TrioTim or Verio MRI scanners (Siemens Medical, Erlangen, Germany). A magnetization-prepared rapid gradient echo (MPRAGE) sequence was used to acquire high-resolution T1-weighted anatomical images (repetition time=2300, echo time=2.98, flip angle=9°, 240 x 256 matrix, 160-192 slices, slice thickness=1.0 mm, voxel size=1x1x1mm^3). The T1 acquisition protocol followed ADNI-3 sequence parameter recommendations: http://adni.loni.usc.edu/wp-content/uploads/2017/07/ADNI3-MRI-protocols.pdf
Detailed description of MR-acquisition protocol can be found in the PPMI MRI Technical Operations Manual: http://www.ppmi-info.org/wp-content/uploads/2017/06/PPMI-MRI-Operations-Manual-V7.pdf

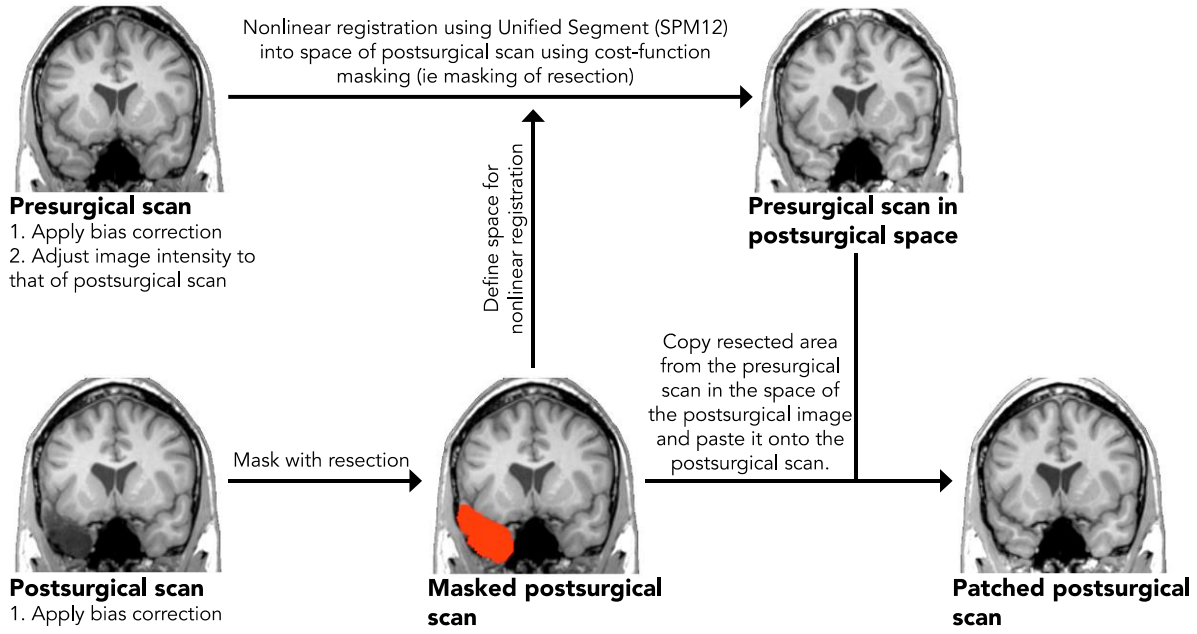
2.3 Southwest University Longitudinal Imaging Multimodal study (SLIM)
Number of volunteers: 53
Reference for Dataset: *Liu W, Wei D, Chen Q, et al. Longitudinal test-retest neuroimaging data from healthy young adults in southwest China. Sci Data 2017; 4: 170017.*
MR-acquisition: 3T Siemens Trio MRI scanner (Siemens Medical, Erlangen, Germany). A magnetization-prepared rapid gradient echo (MPRAGE) sequence was used to acquire high-resolution T1-weighted anatomical images (repetition time = 1900 ms, echo time = 2.52 ms, inversion time = 900 ms, flip angle = 9 degrees, resolution matrix = 256 x 256, slices = 176, thickness = 1.0 mm, voxel size=1x1x1mm^3.

3. MRI preprocessing procedure

3.1 Extraction of resection masks
We extracted surgical resection masks with an automated procedure, as described previously (*Galovic M, Baudracco I, Wright-Goff E, et al. Association of Piriform Cortex Resection With Surgical Outcomes in Patients With Temporal Lobe Epilepsy. JAMA Neurol. 2019;76(6):690-700*). This approach was based on a previously proposed lesion-segmentation algorithm (*Seghier ML, Ramlackhansingh A, Crinion J, Leff AP, Price CJ. Lesion identification using unified segmentation-normalisation models and fuzzy clustering. Neuroimage 2008; 41: 1253-66*).
We used pre- and postsurgical scans in every subject to extract the difference between these two scans using the unified segmentation-normalization procedure implemented in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) with an empirical prior for an atypical tissue class (i.e. the resection). The procedure was iterated twice to refine the resection mask and the mask was nonlinearly coregistered into the space of the presurgical image. All obtained masks were checked by an investigator (MG) and if necessary manually adjusted. To correct for different head sizes, resection volumes were estimated after spatially normalizing them into a standard template with deformations estimated using the presurgical image.

3.2 Spatial normalization
Preprocessing of postsurgical scans can be problematic due to the lack of appropriate normalisation templates and brain shift caused by surgery. We aimed to minimise the impact of the resections using a novel procedure that shares its methodological concepts with "enantiomorphic normalisation", proposed by Nachev and colleagues in 2008. In the enantiomorphic procedure, a unilateral lesion is "patched" by copying and pasting the corresponding area from the contralateral hemisphere. In this manner, the lesion is made to look like healthy brain tissue. Image preprocessing of patched images is more accurate and efficient and does not violate the preprocessing assumptions. After spatial preprocessing the patch is masked, in order not to influence further analyses.
In our study, we obtained presurgical scans for all subjects from the postsurgical group. Thus, we decided to patch the resected area on the postsurgical scan using the corresponding area from the presurgical scan. In this manner, we effectively mimicked the behaviour of presurgical scans during spatial preprocessing.
In order to achieve this (Supplemental Figure 1), we first bias corrected both pre- and postsurgical scans and aligned their image intensities. Next, the postsurgical scan was masked with the resection mask obtained using a previously described procedure (see Online Supplement section 3.1). The masked postsurgical scan was nonlinearly registered into the space of the presurgical image using cost-function masking, as proposed by Brett et al. 2001. Nonlinear registration of pre- and postsurgical images remains highly accurate despite the presence of a lesion, because of the high between-scan similarities acquired in the same subject. Next, we applied the inverse of these deformations to the presurgical scan to transform it into the space of the postsurgical image. Lastly, we copied and pasted a patch corresponding to the resection mask with smoothed edges from the pre- onto the postsurgical scan. This produced a patched postsurgical scan that would behave similarly to the

presurgical scan during image preprocessing. Preprocessing was done using SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/).



Supplemental Figure 1: *Illustration of patching procedure during image preprocessing.*

3.3 Estimation of cortical thickness

All subjects were preprocessed using the same fully automated, validated, and reliable (*see references below*) Computational Anatomy Toolbox (CAT12, http://www.neuro.uni-jena.de/cat/) running in SPM12 (Wellcome Centre for Human Neuroimaging), as described previously (*Galovic M, van Dooren VQH, Postma T, et al. Progressive Cortical Thinning in Patients With Focal Epilepsy. JAMA Neurol. July 2019*).
Cortical thickness was estimated using the projection-based thickness method, that was previously validated using spherical and brain phantoms confirming accurate measurements under a wide set of parameters for several thickness levels (Dahnke *et al.*, 2013). The CAT12 toolbox showed excellent test-retest reliability (R^2 = 0.986) and was validated against other cortical surface reconstruction methods, showing fewer thickness measurement errors than comparable approaches (Righart *et al.*, 2017; Seiger *et al.*, 2018).

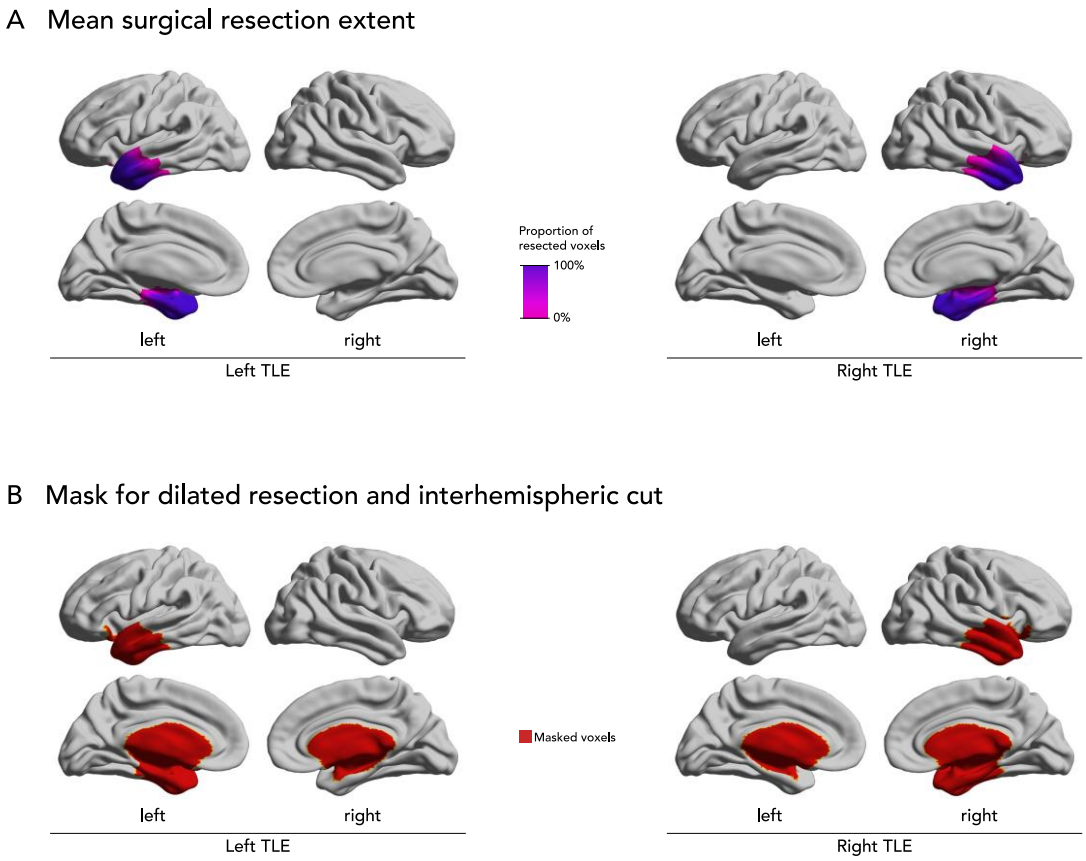
References:

- Dahnke R, Yotter RA, Gaser C. Cortical thickness and central surface estimation. *Neuroimage*. 2013;65:336-348. doi:10.1016/j.neuroimage.2012.09.050.
- Righart R, Schmidt P, Dahnke R, et al. Volume versus surface-based cortical thickness measurements: A comparative study with healthy controls and multiple sclerosis patients. *PLoS ONE*. 2017;12(7):e0179590. doi:10.1371/journal.pone.0179590.
- Seiger R, Ganger S, Kranz GS, Hahn A, Lanzenberger R. Cortical Thickness Estimations of FreeSurfer and the CAT12 Toolbox in Patients with Alzheimer's Disease and Healthy Controls. *J Neuroimaging*. May 2018. doi:10.1111/jon.12521.

All data were quality controlled according to procedures implemented in CAT12 and scans with misalignment, misregistration, or inaccurate thickness estimation were excluded.

3.4 Mean extent of resection and masking

The mean extent of surgical resections is displayed in Supplemental Figure 2A. As a next step, we created a mask including voxels that were resected in at least 10% of subjects and dilated the mask by a safety margin of 3mm. We also included the interhemispheric cut in the mask. Separate masks were created for left and right temporal lobe epilepsy (TLE). The masks were then transformed from voxel space into surface space using commands in the CAT12 toolbox (Supplemental Figure 2B).



Supplemental Figure 2: *(A) Mean extent of standard anterior temporal lobe resection in left and right temporal lobe epilepsy (TLE). (B) Mask created out of dilated resection and interhemispheric cut.*

3.5 Masking of the resection

All postsurgical scans were masked with the individual resection mask dilated by 3mm to remove the patched areas and to apply a safety margin for potential structural alterations in the regions immediately surrounding the resection (Online Supplement section 3.4). Similarly, all presurgical and healthy volunteer scans were masked with the respective left or right temporal mean resection mask with a safety margin of 3mm, when comparisons with postsurgical scans were performed. Cortical thickness maps were smoothed with a 15-mm surface-based kernel.

4. Baseline characteristics in left and right TLE

There were no differences in baseline characteristics between patients with left and right TLE (Supplemental Table 2).

Supplemental Table 2: *Baseline characteristics in patients with left and right TLE*

	Left TLE (n = 47)	Right TLE (n=38)	P value
Gender			
Female	26 (55%)	25 (67%)	0.38
Male	21 (48%)	13 (34%)	
Age			
Age mid-scan *(years)*	40 ± 12	39 ± 12	0.88
Age at seizure onset *(years)*	14 ± 10	15 ± 11	0.77
Age at surgery *(years)*	40 ± 12	40 ± 12	0.99
Duration of epilepsy at surgery *(years)*	26 ± 14	25 ± 13	0.82
Presurgical seizures			
Focal aware	26 (55%)	16 (42%)	0.28
Focal impaired awareness	45 (96%)	37 (97%)	1.00
Focal to bilateral tonic-clonic	37 (79%)	28 (74%)	0.62
Focal impaired awareness frequency *(per month)*	10 ± 9	33 ± 162	0.40
Focal to bilateral tonic-clonic frequency *(per month)*	0.9 ± 2.2	0.4 ± 1.2	0.29
Pathology			
Hippocampal sclerosis	35 (75%)	29 (76%)	1.00
Dysembryoplastic neuroepithelial tumor	6 (13%)	3 (8%)	0.73
Cavernoma	1 (2%)	2 (5%)	0.58
Other	9 (19%)	7 (18%)	1.00
Surgical outcome			
Seizure free after surgery (ILAE Class Ia)	19 (40%)	16 (42%)	0.54
Other			
Number of antiepileptic drugs at surgery	3 ± 1	2 ± 1	0.38
History of a precipitating injury*	5 (11%)	2 (5%)	0.45
History of childhood febrile convulsions	10 (21%)	3 (8%)	0.13
History of depression	15 (32%)	13 (35%)	0.82
History of psychosis	4 (9%)	3 (8%)	1.00
History of anxiety disorder	7 (15%)	5 (14%)	1.00

Data displayed as N (%) or mean ± standard deviation. Data analysed with Fisher's exact test for nominal variables or with the independent T-Test for scalar variables. * Most commonly reported precipitating injuries were a history of meningitis or traumatic brain injury.

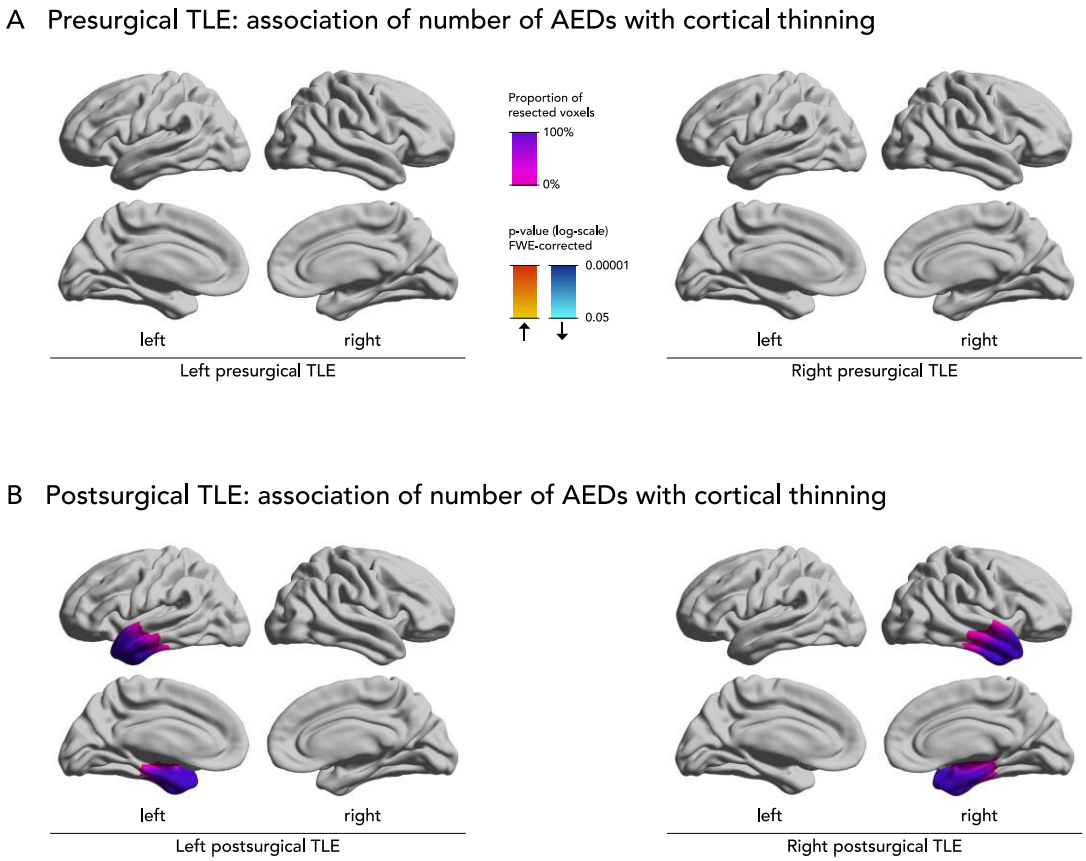
5. Coefficient of variation

The coefficient of variation of cortical thickness measurements was lower in healthy volunteers (4.0%) and in the postsurgical patient group (5.1%) compared to the presurgical patient group (5.8%). The increased variability in epilepsy patients compared to healthy volunteers is most likely explained by biological variability, i.e. differences in severity and duration of epilepsy.

This demonstrates that there was smaller data variability in the healthy volunteer cohort and in the postsurgical cohort than in the presurgical cohort. The greater the variability the lesser the accuracy for detecting cortical thinning: that the presurgical group nonetheless showed *greater* thinning compared to the postsurgical group shows this is highly unlikely to have resulted from differences in variability, for that would cause an effect in the *opposite* direction. Thus, our findings cannot be explained by a reduced sensitivity to detect cortical thinning in postsurgical compared to presurgical epilepsy patients.

6. Association of antiepileptic drugs with cortical thinning

We assessed the association of number of antiepileptic drugs (AEDs) with progressive cortical thinning in both pre- and postsurgical patient groups. We followed a similar statistical procedure as for the analyses in the main manuscript. We did not find any significant association of AED load with cortical thinning before or after surgery (Supplemental Figure 3).



Supplemental Figure 3: *Association of number of AEDs at baseline scan with cortical thinning before (A) or after (B) temporal lobe resection in left and right TLE.*

7. Findings in a presurgical subgroup with short interscan intervals

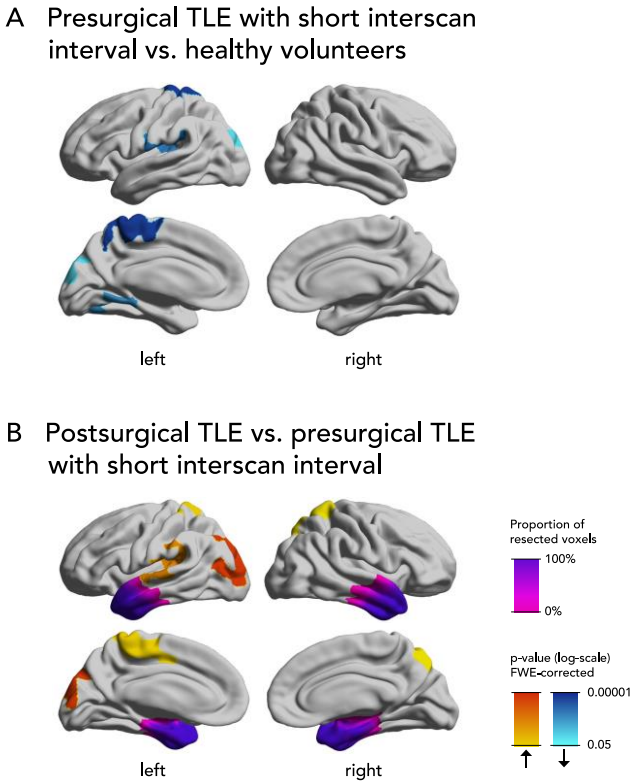
The presurgical group had a longer interscan interval (28 ± 16 months) compared to the postsurgical group (14 ± 11 months). Although all analyses were adjusted for lengths of interscan intervals, we performed a sensitivity analysis in a presurgical subgroup with short interscan intervals.

In this sensitivity analysis, we included presurgical cases with an interval between the scans shorter than 2 years. The 13 cases in this subgroup had an interval of 15 ± 5 months, comparable with the postsurgical group. Because of the small number of subjects we did not split the analyses into left and right TLE. Thus, we compared progressive cortical thinning in the whole subgroup of presurgical patients with short interscan interval against healthy volunteers (Supplemental Figure 4A) and postsurgical cases (Supplemental Figure 4B). We used a bilateral resection mask because this group included both left- and right-sided resections.

Compared to healthy volunteers (Supplemental Figure 4A), the presurgical subgroup had progressive cortical thinning in the left paracentral lobule and superior frontal gyrus (1002 vertices, $p<0.0001$), insular cortex and postcentral gyrus (968 vertices, $p=0.0002$), lingual and fusiform gyri (623 vertices $p=0.0004$), and superior parietal cortex (500 vertices, $p=0.01$).

Comparing the postsurgical group to the presurgical subgroup (Supplemental Figure 4B), there was postoperatively reduced thinning in the left superior and inferior parietal cortex (1659 vertices, $p<0.0001$), left superior temporal and supramarginal gyri and insular cortex (1466 vertices, $p=0.0007$), left paracentral lobule and superior frontal gyrus (1041 vertices, $p=0.01$), and right superior postcentral gyrus and superior parietal cortex (849 vertices, $p=0.02$).

To conclude, we demonstrated in a presurgical subgroup with short interscan intervals progressive cortical thinning compared to healthy controls. We showed that thinning was significantly less postoperatively. Thus, we replicated the key findings of the main study, making it unlikely that the results can be explained by a difference in interscan intervals between the pre- and postsurgical groups.



Supplemental Figure 4: *Comparison of progressive cortical thinning in a subgroup of presurgical cases with short interscan intervals with healthy volunteers (A) and the postsurgical group (B).*