Supplementary materials

Cortical microstructural gradients capture memory network reorganization in temporal lobe epilepsy

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Supplementary methods and results

Quantitative intracortical intensity profiling

We investigated the laminar underpinnings of microstructural gradient reconfigurations using quantitative profiling of intracortical R1 intensities across cortical depths. This approach characterizes the shape of intracortical microstructural profiles by calculating the central moments (mean amplitude, mean depth of peak, SD, skewness, and kurtosis) of intensity values distributed between the pial and white matter boundaries (Supplementary Fig. 5A). These features thus captured subject-specific properties of local laminar myeloarchitecture across the cortex. Indeed, these metrics could differentiate primary and unimodal cortices from heteromodal and paralimbic regions (Supplementary Fig. 5B). Each profile shape map was statistically compared between TLE and HCs using surface-based linear models, while controlling for effects of age and sex (Supplementary Fig. 6A). We then computed spatial correlations between effect size maps associated with each statistical moment and gradient contractions (Supplementary Fig. 6B). Statistical significance was determined using nonparametric spin permutation testing (1,000 permutations) implemented in the BrainSpace toolbox. Targeted post-hoc analyses then compared average microstructural moment changes within significant clusters of gradient contractions in TLE patients relative to controls (Supplementary Fig. 6C). Compared to controls, changes in each central moment could be observed in the TLE group: Although these alterations were not confined to ipsilateral temporal cortices, consistent alterations in all moments could be observed in this region.

Controlling for cortical morphology, volume, and pericortical blurring

We repeated group comparisons in microstructural gradients while additionally controlling for cortical thickness. For this purpose, we fit a vertex-wise linear model predicting individual microstructural gradients from cortical thickness, with residuals serving as estimates of thickness-corrected microstructural gradients. 1 Controlling for cortical thickness at the vertex-level had little impact on group differences in gradient scores (Supplementary Fig. 7A). In line with initial findings, correcting individual gradient maps for vertex-wise measures of cortical thickness revealed significant microstructural gradient contractions in ipsilateral temporopolar and lateral
prefrontal regions ($p_{\text{FWE}} < 0.001$). Controlling for estimates of regional volume yielded similar findings, showing significant microstructural gradient contractions in ipsilateral temporopolar and frontopolar regions ($p_{\text{FWE}} < 0.001$; Supplementary Fig. 7B). We additionally controlled for grey-white matter interface blurring (Supplementary Fig. 7C). We estimated blurring from qT1 intensity gradients computed perpendicular to the cortical mantle, in line with previous work.\(^1\-^3\) The same vertex-level procedure as for cortical thickness and volume was applied to correct for cortex-wide measurements of cortical blurring. Results were also consistent with initial findings after correcting for vertex-wise grey-white matter interface contrast measured from qT1 images.

**Controlling for cerebrospinal fluid partial volume effects**

We repeated group comparisons in microstructural gradients while additionally controlling for individual-specific assessments of cerebrospinal fluid partial volume effects (CSF-PVE). Volumetric CSF-PVE maps were obtained by applying FSL-FAST\(^4\) to non-uniformity corrected T1-weighted images. Probabilistic CSF-PVE maps underwent an affine transformation to each participant’s native Freesurfer space and were sampled at the midpoint between the pial and white matter boundaries (midthickness surface). Resulting surface-mapped CSF-PVE estimates were then resampled to the conte69 surface template, and downsampled to a density of 5,000 vertices per hemisphere. As for control analyses involving cortical thickness, volume estimates, and cortical interface blurring, we fit a vertex-wise linear model predicting individual microstructural gradients from CSF-PVE sampled at midthickness, with residuals serving as estimates of PVE-corrected microstructural gradients. Controlling for CSF-PVE at the vertex-level had little impact on group differences in gradient scores, showing significant microstructural gradient contractions in ipsilateral temporopolar and prefrontal regions ($p_{\text{FWE}} < 0.001$; Supplementary Fig. 8).
### Supplementary table 1: Clinical characteristics and patient demographics

| ID   | Age | Sex | HD | Laterality | MRI diagnosis                                      | Surgery       | Engel outcome |
|------|-----|-----|----|------------|---------------------------------------------------|---------------|---------------|
| PX002| 24  | M   | R  | R          | T2/FLAIR hyperintensity in hippocampus             | NA            | NA            |
| PX003| 54  | M   | R  | L          | T2/FLAIR hyperintensity in hippocampus             | NA            | NA            |
| PX005| 26  | F   | R  | L          | MRI-negative                                       | RFTC          | 1a            |
| PX008| 20  | F   | R  | L          | Tumour in the uncus                                | Tumour resection | 1a            |
| PX012| 33  | F   | R  | L          | MTS                                                | NA            | NA            |
| PX013| 57  | M   | R  | L          | MTS                                                | SAH           | 1a            |
| PX019| 31  | M   | R  | L          | Suspected FCD in parahippocampal gyrus             | NA            | NA            |
| PX021| 55  | F   | R  | L          | T2/FLAIR hyperintensity in hippocampus             | SAH           | 1a            |
| PX025| 25  | M   | R  | R          | T2/FLAIR hyperintensity in hippocampus             | NA            | NA            |
| PX027| 40  | F   | L  | L          | MRI-negative                                       | NA            | 1a            |
| PX028| 22  | F   | R  | R          | MRI-negative                                       | RFTC; CAH     | 1a            |
| PX032| 53  | F   | R  | L          | MTS                                                | NA            | NA            |
| PX036| 24  | M   | R  | L          | Cystic malformation and FCD                        | NA            | 1a            |
| PX042| 38  | M   | R  | L          | MRI-negative                                       | CAH           | 1a            |
| PX043| 37  | F   | R  | R          | MTS                                                | NA            | NA            |
| PX044| 43  | M   | R  | R          | MTS                                                | NA            | NA            |
| PX047| 35  | M   | R  | L          | MRI-negative                                       | CAH           | 1a            |
| PX050| 32  | M   | R  | R          | MRI-negative                                       | RFTC          | 1a            |
| PX053| 41  | M   | R  | L          | T2/FLAIR hyperintensity in mesio-temporal lobe and insula | RFTC          | 4             |
| PX055| 27  | F   | R  | L          | MRI-negative                                       | NA            | NA            |
| PX062| 44  | M   | R  | L          | MTS                                                | CAH           | 1a            |

Detailed clinical information of discovery cohort. Abbreviations: CAH: cortico-amygdalohippocampectomy; FCD: Focal cortical dysplasia; HD: Handedness; MTS: mesio-temporal sclerosis; RFTC: stereotactic radiofrequency thermocoagulation; SAH: selective amygdalo-hippocampectomy
### Supplementary table 2: Genes included in disease-related transcriptomics analysis

| Disorder                                    | Genes                                      |
|---------------------------------------------|---------------------------------------------|
| All epilepsies                              | BCL11A, BRD7, FANCL, HEATR3, SCN1A, SCN2A, SCN3A, TTC21B |
| Childhood absence epilepsy                  | BCL11A, FANCL, ZEB2                         |
| Focal epilepsy                              | SCN1A, SCN2A, SCN3A, TTC21B                 |
| Focal epilepsy (hippocampal sclerosis)      | C3orf33, GJA1, KCNAB1, SLC33A1              |
| Generalized epilepsy                        | ATXN1, BCL11A, FANCL, GABRA2, GRIK1, KCNN2, PCDH7, PNPO, SCN1A, SCN2A, SCN3A, STAT4, TTC21B |
| Juvenile myoclonic epilepsy                 | STX1B                                      |

**Table S2.** List of disease-specific risk genes used in the present study extracted from previously published GWAS. Gene lists and pre-processed gene expression data are openly available as part of the ENIGMA toolbox.
Supplementary figure 1

Supplementary figure 1. Post hoc analysis of microstructural profile similarity patterns. (A) The top panel shows the microstructural similarity profile of vertices located in the temporopolar cluster for the HC group (average of vertices in the cluster, which is outlined in white). Compared to controls, the TLE group showed reduced microstructural similarity to paralimbic and transmodal cortices (left, significant regions outlined in white, $p_{\text{FWE}} < 0.025$), and higher microstructural profile similarity to unimodal sensory and motor regions (right, significant regions outlined in white, $p_{\text{FWE}} < 0.025$). (B) The top panel shows the microstructural similarity profile of vertices located in the prefrontal cluster for the HC group (average of vertices in the cluster, which is outlined in white). Compared to controls, the TLE group showed reduced microstructural similarity to paralimbic and transmodal cortices (left, significant regions outlined in white, $p_{\text{FWE}} < 0.025$), and higher microstructural profile similarity to unimodal sensory and motor regions (right, significant regions outlined in white, $p_{\text{FWE}} < 0.025$).
Supplementary figure 2

(A) To assess inter-individual consistency in sensory-fugal gradient topography, we correlated each participant’s gradients (G1-G10) with the G1 template used for subject alignment. For aligned and unaligned data, each participant’s G1 showed the strongest correlation with G1 (the sensory-fugal gradient) on the template. Correlation coefficients (Spearman’s r) between each participant’s own G1 and the G1 template are displayed in box/dot plots stratified by group (B) The variance explained by G1 was higher in HC than TLE (T= -2.752; \(p=0.008\)). (C) We repeated case-control comparisons of microstructural gradient scores while additionally controlling for the proportion of explained variance accounted for by G1 in each subject. The overall topography of gradient reorganizations strongly resembled original findings, although effects in the ipsilateral prefrontal lobe only reached trend-level statistical significance after correction for multiple comparisons (\(p_{FWE}=0.054\)). Gradient reductions in ipsilateral temporopolar regions remained statistically significant (\(p_{FWE}<0.001\)).
Supplementary figure 3

A | Subject-level effects

Supplementary figure 3. Patient-level consistency of microstructural gradient contractions and asymmetry. (A) 80.95% of patients showed moderately negative average z-scores (z<1) in either significant cluster of gradient reductions (57.14% at z<1.5, 33.33% at z<2). After correction for age and sex, we observed strong reductions in gradient scores in TLE patients compared to HCs in both temporal lobe (cohen’s d=-2.298) and prefrontal clusters (cohen’s d=-1.995). (B) Microstructural gradient contractions also showed potential for lateralization of the seizure focus in TLE. Indeed, 90.48% of patients showed lower average z-scores in the ipsilateral vs. contralateral hemisphere in either significant cluster of gradient reductions. When considering each cluster independently, this proportion remained high, with 76.14% of patients showing lower average z-scores in the ipsilateral temporal (cohen’s d=-0.638) or prefrontal cluster (cohen’s d=-0.688) relative to its homologous region in the contralateral hemisphere.
Supplementary figure 4

**Supplementary figure 4. Topography of TLE and HC microstructural gradients 1-5.** (A) Sample-wide (including all TLE and HC participants) gradient templates (G1-G5). (B) Differences in gradient scores between TLE and HCs are shown in unthresholded t-statistic maps, with significant clusters of findings outlined in white ($p_{FWE}<0.025$) and trend-level effects outlined in thinner grey clusters ($p_{FWE}<0.1$). As in our main analyses, all group comparisons corrected for age and sex. Anterior temporal regions ipsilateral to the seizure focus were systematically perturbed in TLE relative to corresponding gradients in HCs.
Supplementary figure 5. **Group-level central moment maps.** (A) Intensity profiles sampled at a single vertex in the anterior cingulate cortex (ACC, green) and primary visual cortex (V1, blue), averaged across all healthy control participants. The mean amplitude of each profile is indicated by the dashed line, and was computed by averaging the intensity values sampled at each depth for each vertex. The shape of each profile could be quantified by first converting each profile to a frequency distribution: The corresponding frequency distribution of the ACC and V1 profiles (left) are displayed in a histogram (right). We computed the mean (m1), standard deviation (m2), skewness (m3), and kurtosis (m4) of depth-dependent R1 intensity distributions, thus capturing the overall shape of vertex-wise microstructural profiles along the cortical sheet. (B) Central moment maps were averaged across all subjects and projected to the cortical surface. In line with previous work, these metrics could differentiate primary and unimodal cortices from heteromodal and paralimbic regions.
Supplementary figure 6

**A | Quantitative intracortical intensity profiling**

Mean amplitude

**B | Association to gradient contractions**

| m1 | m2 | m3 | m4 |
|---|---|---|---|
| r=0.249; p=0.014 | r=0.134; p=0.189 | r=0.854; p<0.001 | r=-0.077; p=0.473 |
| r=-0.834; p<0.01 |

**C | Post hoc analysis**

| Mean amplitude | m1 | m2 | m3 | m4 |
|---|---|---|---|---|
| d=0.717 | d=0.628 | d=-1.806 | d=1.795 |
| d=-0.207 | d=-0.071 | d=1.419 | d=1.199 |

**Supplementary figure 6.** Perturbed laminar microarchitecture underlies sensory-fugal gradient reorganizations in TLE. (A) The shape of intracortical R1 profiles was quantified using the mean amplitude and four central moments, calculated across cortical depths at each vertex. After correction for age and sex, differences between TLE and HC groups could be seen across all moments (only effect sizes > 0.5 and < -0.5 are shown) (B) Spatial correlations between central moment and gradient change maps showed strongest cortex-wide correlations with m2 (standard deviation) and m4 (kurtosis). Statistical significance was determined using nonparametric spin permutation tests. (C) *Post hoc* analyses targeting significant clusters of gradient reductions highlighted more severe perturbations in laminar architecture in ipsilateral temporopolar (left) relative to prefrontal regions (right). Larger black points and associated lines on each subplot represent median as well as 25th and 75th percentile of group distributions.
Supplementary figure 7. Microstructural gradient reorganizations in TLE are robust to altered cortical thickness, volume, and blurring. (A) Patients showed widespread and bilateral patterns of cortical thinning relative to controls, with stronger effects seen in mesio-temporal, frontal, and occipital cortices. Group comparisons of microstructural gradient scores controlling for vertex-wise cortical thickness were consistent with initial findings. (B) Regional volume estimates provided by Freesurfer showed widespread reductions in patients relative to controls predominantly affecting temporal and frontal cortices. Group comparisons of microstructural gradient scores controlling for vertex-wise cortical volume were consistent with initial findings. (C) Subtle reductions in gray/white matter interface contrast were seen bilaterally in temporal and frontal regions in TLE. This metric captured distance-dependent differences in R1 intensity between superficial white matter and deep intracortical intensities. Group comparisons of microstructural gradient scores controlling for vertex-wise contrast alterations were also consistent with initial findings.
Supplementary figure 8

Relation to cerebrospinal fluid (CSF) partial volume effects (PVE)

Supplementary figure 8. Microstructural gradient reorganizations in TLE are robust to CSF PVE. Relatively higher CSF-PVE were seen in lateral temporo-parietal cingulate, and mesial frontal regions. Consistent with patterns of cortical thinning in patients, the TLE group showed rather widespread increased susceptibility to CSF-PVE in insular, parietal, cingulate, and mesio-temporal areas. Group comparisons of microstructural gradient scores controlling for vertex-wise CSF-PVE were consistent with initial findings.
Supplementary figure 9. Replicability of microstructural gradient changes in validation cohort. (A) As in the discovery cohort, quantitative T1 imaging was used as a proxy of intracortical myeloarchitecture in TLE and HC individuals (i). Intensities were sampled at 14 cortical depths between the pial and white matter boundaries, yielding vertex-wise microstructural intensity profiles. Mean R1 (1/T1) intensity calculated across cortical depths is displayed on the surface template. Profiles sampled in the primary visual cortex (V1, blue) and anterior cingulate cortex (ACC, green) showed different shapes in the validation cohort (ii). (B) MPC matrices were constructed by cross-correlating vertex-wise intensity profiles using partial correlations controlling for the average cortex-wide profile, and normalized angle affinity matrices were generated from corresponding subject-level MPC matrices (iii). We applied diffusion map embedding to identify eigenvectors (gradients) describing main axes of variance in inter-regional similarity of cortical microstructural patterns. G1 is shown in (iv) for HC and TLE groups. Subject-specific gradients were aligned to the previously computed template derived from the discovery cohort data. (C) Surface-based linear models controlling for age and sex revealed significant differences in gradient scores between TLE and HC groups. Patients showed significant reductions in gradient scores in ipsilateral mesial and inferior anterior temporal lobe regions ($p_{FWE}<0.001$).
Supplementary figure 10. Replicability of microstructural gradient changes and neural contextualization findings when pooling discovery and validation cohorts. (A) The principal microstructural gradient strongly differentiated sensory-motor cortices from limbic and paralimbic regions in both cohorts, and both HCs and TLE groups. Qualitatively, lateral frontal areas were situated further from the paralimbic apex of the gradient in the validation cohort compared to the discovery cohort. (B) Surface-based linear models pooling both cohorts and controlling for age, sex, and site revealed significant differences in gradient scores between TLE and HC groups. Patients showed significant reductions in gradient scores in ipsilateral mesial and inferior anterior temporal lobe regions ($p_{FWE}<0.001$). Trend-level effects were seen in contralateral mesiotemporal regions (TLE < HC; $p_{FWE}=0.070$), as well as ipsilateral
paracentral (TLE > HC; $p_{FWE} = 0.053$) and contralateral occipital areas (TLE > HC; $p_{FWE} = 0.044$) (C) The topography of microstructural gradient changes in TLE obtained from this pooled-cohort analysis was significantly correlated with the sensory-fugal (S-F) pattern of cytoarchitectural differentiation ($r = -0.513; p_{spin} = 0.006$; top row), but not with the anterior-posterior (A-P) axis ($r = -0.242; p_{spin} = 0.426$; bottom row). (D) TLE-related gradient changes also followed the average expression patterns of genes related to hippocampal sclerosis ($r = -0.453; p_{spin} = 0.012$). No significant correlations were found with other epilepsy type-related gene expression patterns (distribution of correlations obtained from null model, from top to bottom: All epilepsies: $r = 0.175, p_{spin} = 0.635$; Focal epilepsy: $r = 0.337, p_{spin} = 0.122$; IGE: $r = 0.182, p_{spin} = 0.566$; JME: $r = 0.422, p_{spin} = 0.077$; CAE: $r = -0.011, p_{spin} = 0.973$; Focal HS: $r = -0.453, p_{spin} = 0.012$). Statistical significance of correlations with histological and transcriptomic features was determined using non-parametric spin permutation testing (1,000 permutations).
Supplementary figure 11. **Epilepsy syndrome gene co-expression maps and relationship to TLE-related microstructural gradient reorganizations.** Cross-referencing results of recent genome-wide association (GWAS) studies with data from the Allen Human Brain Atlas (AHBA) provided distinct gene co-expression patterns for several epilepsy syndromes (left). Only the correlation between co-expression patterns of genes associated with focal hippocampal sclerosis and microstructural gradient reorganizations seen in our TLE sample reached statistical significance after controlling for shared spatial autocorrelation. Scatter plots (right) depict the spatial relationship between each epilepsy syndrome (y-axis) and gradient changes (x-axis).
Supplementary figure 12

A | Temporopolar seed intrinsic connectivity

B | Prefrontal seed intrinsic connectivity

Supplementary figure 12. Intrinsic functional connectivity changes associated with microstructural gradient seeds. (A) Results of seed-based functional connectivity analysis during a resting-state acquisition, centered on peak regions in the temporopolar cluster of gradient contractions. Connectivity patterns in HCs are displayed in the top portion of the panel. Compared to HCs, TLE patients showed reduced connectivity to nodes of the default mode network, with strongest effects in the ipsilateral precuneus, as well as distributed connectivity increases with the anterior insula, lateral occipital regions, and fronto-parietal regions. (B) Results of seed-based functional connectivity analysis during a resting-state acquisition, centered on peak regions in the prefrontal cluster of gradient contractions. Connectivity patterns in HCs are displayed in the top portion of the panel. Compared to HCs, TLE patients showed reduced connectivity to distributed fronto-parietal regions, as well as lateral temporal cortices. In both A and B, Cohen’s $d$ maps are thresholded to show effect sizes larger than 0.5 and lower than -0.5. Connectivity between both seeds did not significantly differ between patients and controls in ipsilateral ($t_{-1.266;} d_{-0.434}; p_{0.212}$) and contralateral ($t_{-0.963;} d_{-0.334}; p_{0.340}$) hemispheres.
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