Epileptogenic tubers are associated with increased stereo-electroencephalography derived functional connectivity in tuberous sclerosis complex

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Abstract
Objective: Epileptic spasms (ES) are common in tuberous sclerosis complex (TSC). However, the underlying network alterations and relationship with epileptogenic tubers are poorly understood. We examined interictical functional connectivity (FC) using stereo-electroencephalography (SEEG) in patients with TSC to investigate the relationship between tubers, epileptogenicity, and ES.

Methods: We analyzed 18 patients with TSC who underwent SEEG (mean age = 11.5 years). The dominant tuber (DT) was defined as the most epileptogenic tuber using the epileptogenicity index. Epileptogenic zone (EZ) organization was
Epileptic spasms (ES) are a common seizure type in tuberous sclerosis complex (TSC), occurring in approximately 40%-50% of patients. ES most commonly, but not exclusively, begin in infancy, and TSC is one of the most common causes of West syndrome, a syndrome that comprises ES, hypsarrhythmia, and developmental regression of variable degree.

ES are associated with TSC2 mutations, more epileptogenic radiological tuber types, increased tuber burden, current and progressive cognitive impairment, autism, and development of an epileptic encephalopathy. There is also a close relationship between ES and poor neurocognitive development. There is now considerable interest in identifying biomarkers predicting the development of ES and neurocognitive impairment in TSC that can guide early presymptomatic treatment.

The exact mechanism of ES and their prevalence in TSC are incompletely understood. However, there is evidence suggesting that diffuse alterations to functional connectivity (FC) may be a key characteristic of ES. Supporting this hypothesis is a recent lesion mapping study in children with TSC, which found that strong negative FC to bilateral globi pallidi and cerebellar vermes predicts ES rather than any specific tuber location. Two scalp electroencephalographic (EEG) studies have provided separate evidence for ES and developmental delay being a state of excessive global connectivity. Yet, despite these widespread network alterations, it is well established that focal lesions, including tubers, can generate ES, and that focal lesionectomy can lead to long-term seizure freedom. Overall, the role of tubers, particularly the most epileptogenic tubers, in these ES-generating networks is poorly understood.
Stereo-EEG (SEEG) is well suited to addressing this knowledge gap. Simultaneous sampling of multiple tubers and normal-appearing cortex across multiple lobes allows an interrogation of tuber epileptogenicity, epileptogenic networks, and FC alterations. Using SEEG, we have recently described two distinct spatial epileptogenic zone (EZ) organizations in TSC: a focal tuber organization, where the EZ is limited to a single dominant tuber; and a complex organization, where the EZ involves multiple tuber and nontuber structures.

In this current study, we examined interictal FC using SEEG data in a cohort of patients with TSC who underwent presurgical SEEG. We hypothesized that (1) patients with ES as a current seizure type will have increased interictal FC compared to those without ES, particularly in brain networks involving the most epileptogenic tuber; and (2) that the two distinct TSC EZ organizations would have unique FC patterns, specifically, that patients with a focal pattern would exhibit weak connections to nearby tubers and cortex, allowing it to exist as a relatively isolated epileptogenic network.

2 | MATERIALS AND METHODS

2.1 | Case selection

Patients with a genetic or clinical diagnosis of TSC who underwent SEEG between 2004 and 2018 were identified from four French tertiary epilepsy centers. No patients had undergone previous surgery at the time of SEEG. Medical records were reviewed in all patients. The study was approved by the local ethics committee of Lyon’s University Hospital. All patients were included in a recent study examining epileptogenicity in TSC.

2.2 | Radiological tuber classification

Preoperative magnetic resonance imaging (MRI) was examined, and each supratentorial tuber was classified as type A, B, or C, as previously described. Type A tubers are fluid-attenuated inversion recovery (FLAIR) hyperintense and T1 isointense, type B tubers are homogenously FLAIR hyperintense and T1 hypointense, and type C tubers have a FLAIR hypointense center surrounded by a hyperintense rim and are T1 hypointense.

2.3 | SEEG recordings

Following a thorough noninvasive workup, a decision to proceed to SEEG was made at an individual patient level when an electroclinical hypothesis based on noninvasive data had to be verified with intracerebral recordings. Intracerebral multicontact electrodes (5–18 contacts, diameter = .8mm, 1.5mm apart) were implanted according to Talairach’s stereotactic method or using a frameless stereotactic surgical robot.

All seizures recorded during SEEG were reviewed. ES were defined as per the 2017 International League Against Epilepsy (ILAE) classification: “A sudden flexion, extension, or mixed extension–flexion of predominantly proximal and truncal muscles that is usually more sustained than a myoclonic movement but not as sustained as a tonic seizure. Limited forms may occur: Grimacing, head nodding, or subtle eye movements.” In keeping with this guideline, ES, rather than infantile spasms, can occur at all ages. Prior Infantile ES Syndrome was defined as the presence of infantile onset spasms and developmental delay and/or hypsarrhythmia. This definition encompasses infants with West syndrome and is consistent with the Infantile ES Syndrome definition proposed by the ILAE.

2.4 | Anatomical regions of interest and EZ organization

As previously described, for each patient, a subset of SEEG channels were selected based upon their anatomical location within tuber or normal-appearing gray matter. A maximum of 10 electroclinical seizures were selected for each patient, including ES in some patients. The epileptogenicity index (EI) was then calculated for all seizures within this subset of selected SEEG channels using Anywave Software (available at http://meg.univ-amu.fr/wiki/AnyWave). The median EI for each channel across all seizures for an individual patient was calculated. The dominant tuber was then defined as the tuber with the highest median EI, nearby cortex was defined as the normal-appearing cortex in the same lobe as the dominant tuber, and distant cortex was defined as the normal-appearing cortex in all other lobes.

In 13 patients with adequate tuber sampling, the EZ organization was defined at a patient level. According to the previous study, the EZ organization was separated into (1) focal tuber (dominant tuber with median EI > .3 and all other regions with median EI ≤ .3) and (2) complex (all other patterns). A detailed description regarding the noninvasive workup, SEEG recordings, anatomical regions of interest, and epileptogenicity analysis can be found in the recently published article from our group.

2.5 | Interictal analysis

For all 18 patients, a 20-min bipolar interictal recording was selected that fulfilled the following criteria: (1)
recorded in a restful, nonsleep state between 8 a.m. and 2 p.m.; (2) not artifact degraded; (3) at least 2 h following or preceding a seizure; and (4) at least 48 h after electrode implantation. The first available segment that fulfilled these criteria was chosen for analysis. Spike rates per bipolar channel were calculated in Anywave using Delphos (Detector of ElectroPhysiological Oscillations and Spikes), which automatically detects spikes.28,29

2.6 | FC analysis

Interictal FC was examined during the 20-min interictal recording, using published methodology.30 One bipolar channel was used for each sublobar or tuber region of interest. We estimated FC with nonlinear regression, \( h^2 \).31,32 Given that neural networks in epilepsy exhibit nonlinear dynamics,31,33–35 this established nonlinear regression technique is recognized as an effective method for quantifying FC, particularly with SEEG signals.30,36 This method quantifies the correlation of the signal amplitude between two channels. \( h^2 \) is similar to \( r^2 \) in linear regression and has a value between 0 (no correlation) and 1 (maximal correlation).

We calculated \( h^2 \) values using Anywave open-source software27 using the following parameters: 8-s sliding window, steps of 4 s, and a maximum lag of .1 s. All \( h^2 \) values were calculated using broadband signals (no low pass, no high pass). The median value of \( h^2 \) across all time windows was calculated for a pair of channels. We computed the median \( h^2 \) between all channels in the same zone to determine intrazone FC and between all channels of two zones to determine interzone FC (see below for the definitions of zones).

2.7 | Anatomical zones: Intrazone and interzone FC

Anatomical zones were defined for FC analysis (Figure 1). If more than two channels were in the same brain area
or tuber, we selected the channel with least artifact and greatest signal amplitude.

Three zones were defined for the intrazone analysis: (1) all tubers, containing one representative bipole from all tubers, including the dominant tuber (5.1 ± 3.8 per patient); (2) nearby cortex (NC), containing all selected bipoles from the subset (5.5 ± 4.8 per patient); and (3) distant cortex (DC), containing all selected bipoles from the subset (7.5 ± 4.4 per patient).

In a second analysis, tuber bipole were subdivided into those sampling the dominant tuber (DT) and other tubers (OT). Six interzone analyses were then performed between the following zones: (1) DT and OT, (2) DT and NC, (3) DT and DC, (4) OT and NC, (5) OT and DC, and (6) NC and DC.

The distance between all bipolar channels selected for FC analyses were calculated given the known relationship between distance and strength of coupling. The Euclidian distance between the center of the two selected contacts of each bipolar channel was calculated using coordinates extracted from inhouse coregistration software. For each pair of bipolar channels being analyzed, we also calculated the mean spike rate of the two bipoles, the epileptogenicity (scored as 1 if at least one bipole had a median EI of >.3, scored as 0 if no bipole had an EI of >.3), and the EZ organization (classified at the patient level as focal or complex).

2.8 Statistical analysis

A Kolmogorov–Smirnov test was performed to test whether continuous variables were normally distributed. Normally distributed continuous variables were presented as mean ± SD, whereas nonparametric continuous variables were presented as median (interquartile range).

Chi-squared and Fisher exact test were utilized to compare categorical data between patients with and without ES. Mann–Whitney U test and independent t-test were utilized to compare continuous variables between patients with and without ES.

The main objective of statistical analyses was to determine whether FC $h^2$ was dependent on several clinical features at the patient level (presence of spasms, number of type C tubers, total number of tubers, age at SEEG, and EZ organization), the type of anatomical connections, and their epileptogenicity. Because spike rate and distance between zones may influence connectivity, those two factors were considered in the model as covariates. To predict the FC $h^2$, we fitted a generalized linear mixed model (GLMM) with gamma log description of the error distribution. The dataset was split into intrazone and interzone connections, and separate GLMMs were performed in each. GLMMs were fitted to the data using lme4 as well as the afex packages for the statistical programming language R. The main advantage of these models is that they are able to handle missing data, complex unbalanced designs, heteroscedasticity, sphericity (correlation structure), and normality violation. A characteristic of GLMMs is that they contain a fixed and random effects structure. Usually, the fixed effects structure estimates the explanatory variables, whereas the random effects structure estimates the stochastic variability.

We defined nine main fixed effects: epileptogenicity (two levels, bipole level), ES as a current seizure type (two levels, patient level), the total of number tubers (numeric value, patient level), the number of type C tubers (numeric value, patient level), EZ organization (two levels, patient level), age at SEEG (numeric value, patient level), distance between bipoles (numeric value, bipole level), spike rate (numeric value, bipole level), and anatomical zone (three intrazone or six interzone levels). The fixed interaction between anatomical zone and ES was also analyzed. The equation of this model is as follows:

$$H2 \sim (\beta_0 + \beta_{S,0}) + \beta_1(\text{Organisation}) + \beta_2(\text{Distance}) + \beta_3(\text{Spike. Rate}) + \beta_4(\text{C. Tubers}) + \beta_5(\text{Total. Tubers}) + \beta_6(\text{Age}) + \beta_7(\text{EZ}) + \beta_8(\text{Anatomy}) + \beta_9(\text{Spasm}) + \beta_{10}(\text{Anatomy: Spasm}) + \varepsilon$$

where $s$ is subject (1:n) and $\varepsilon$ the error. We fit the model with the REML estimation method (restricted maximum likelihood).

Post hoc investigation of significant interactions was achieved on the GLMM using the framework provided by emmeans. Probability values were adjusted using the false discovery rate method to control from multiple comparisons. A $p$ value < .05 was deemed significant.

3 RESULTS

3.1 Baseline characteristics

Eighteen patients with TSC were examined (Table 1). Individual patient characteristics are presented in the supplementary material (Table S1) and have also previously been described. Thirteen patients had sufficient tuber sampling to determine the EZ organization; a focal tuber organization in seven and complex organization in six patients. Nine patients had a prior diagnosis of infantile ES syndrome. Six patients had ES as a current seizure type at the time of SEEG (ES group) and four additional patients had prior ES that had resolved by the time of SEEG. Of the patients with current ES, all had at least one additional seizure type documented in the medical record or recorded during EEG monitoring. Patients with ES had a greater number of C tubers ($p < .001$) and sampled tubers ($p = .035$). However, there was no difference
There was a trend for the ES group to be younger at SEEG (6.2 vs. 20.6 years). No TSC1 mutations were observed in patients with ES as a current seizure type (0/5). Notably, the ES were seen equally across patients with a focal and complex EZ organization. Sixteen patients underwent subsequent resective surgery; 60% and 50% achieved Engel I outcome in the non-ES and ES groups, respectively.

### 3.2 Predictors of intrazone FC

The factors associated with FC ($h^2$) were first examined across all intrazone connections (all tubers, NC, and DC; Table 2). The optimal multivariate model with the best fit was chosen according to Bayesian Information and Akaike Information (BI-AI) criteria and with acceptable conditional $R^2$ (.466), Hosmer–Lemeshow goodness-of-fit test ($p = 1$), and intraclass correlation (ICC; .063) and normal residuals.

Interictal FC ($h^2$) was independently associated with shorter distance between bipoles ($df = 14$, chi-squared = 35.7, $p < .001$), increased spike rate ($df = 14$, chi-squared = 25.6, $p < .001$), and the presence of ES as a current seizure type ($df = 14$, chi-squared = 4.6, $p = .033$; Figure 2A). FC was not associated with the patient level EZ organization and the bipolar level epileptogenicity biomarker (EI).

The anatomical zone–ES interaction variable was significantly associated with $h^2$ ($df = 13$, chi-squared = 7.9, $p = .020$). We proceeded to perform post hoc analyses to

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**Table 1** Baseline characteristics

| Variable                        | Total | No ES | ES  | $p$  |
|---------------------------------|-------|-------|-----|------|
| $n$                             | 18    | 12    | 6   |      |
| Female                          | 9 (50%) | 6 (50%) | 3 (50%) | .99 |
| Age at SEEG, years              | 11.5 [5.6–29.7] | 20.6 [7.5–30.5] | 6.2 [4.1–9.2] | .112 |
| Epilepsy duration, years        | 6.7 [4.1–15.6] | 12.5 [5.3–20.7] | 4.3 [3.3–6.2] | .112 |
| Genetic variant                 |       |       |     |      |
| TSC1                            | 5 (28%) | 5 (42%) | 0 (0%) | .308 |
| TSC2                            | 6 (33%) | 4 (33%) | 2 (33%) |       |
| NMD                             | 1 (6%)  | 0 (0%)  | 1 (17%) |     |
| Not tested                      | 6 (33%) | 3 (25%) | 3 (50%) |       |
| Infantile ES Syndrome           | 9 (50%) | 4 (33%) | 5 (83%) | .306 |
| Additional seizure types        |       |       |     |      |
| FAS                             | 7 (39%) | 6 (50%) | 1 (17%) | .525 |
| FIAS                            | 18 (100%) | 12 (100%) | 6 (100%) | >.99 |
| FBTCS                           | 8 (44%) | 7 (58%) | 1 (17%) | .809 |
| EZ organization                 |       |       |     |      |
| Focal                           | 7 (39%) | 4 (33%) | 3 (50%) | .99* |
| Complex                         | 6 (33%) | 3 (25%) | 3 (50%) |       |
| Insufficient sampling           | 5 (28%) | 5 (42%) | 0 (0%) |     |
| Total tubers                    | 16.3±9.0 | 13.0±7.7 | 23.0±8.1 | .090 |
| Radiological C tubers           | 1.5 [0.0–5.0] | 1.0 [0.0–1.8] | 6.5 [2.8–14.3] | <.001 |
| Tubers sampled                  | 5.4±3.5  | 3.9±2.9  | 8.5±2.5 | .035 |
| Bilateral implantation          | 14 (78%) | 9 (75%) | 5 (83%) | .99 |
| Surgery                         |       |       |     |      |
| Tuberectomy                     | 7 (39%) | 5 (42%) | 2 (33%) | .99 |
| Corticectomy                    | 5 (28%) | 3 (25%) | 2 (33%) |     |
| Lobar [ATL, disconnection]      | 4 (22%) | 2 (17%) | 2 (33%) |     |
| No surgery                      | 2 (11%) | 2 (17%) | 0 (0%) |     |
| Engel I outcome                 | 9/16 (56%) | 6/10 (60%) | 3/6 (50%) | .99 |

*Note: Continuous variables presented as median [interquartile range] or mean±SD.*

*Abbreviations: ATL, anterior temporal lobectomy; ES, epileptic spasms; EZ, epileptogenic zone; FAS, focal aware seizures; FBTCS, focal to bilateral tonic–clonic seizures; FIAS, focal impaired awareness seizure; NMD, no mutation detected.*

*Focal vs. complex.*
**Table 2** Multivariate predictors of intrazone functional connectivity

| Variable                          | Variable information     | Level     | df | \( \chi^2 \) | \( p \) |
|-----------------------------------|--------------------------|-----------|----|---------------|--------|
| Age at SEEG                       | Years                    | Patient   | 14 | .944          | .331   |
| Number of type C tubers           | Number                   | Patient   | 14 | .744          | .388   |
| Number of total tubers            | Number                   | Patient   | 14 | .002          | .961   |
| Distance between bipoles          | Euclidean distance       | Bipole    | 14 | 35.706        | <.001  |
| Spike rate                        | Mean between bipoles     | Bipole    | 14 | 25.757        | <.001  |
| EZ organization                   | Focal vs. complex        | Patient   | 14 | .124          | .725   |
| Epileptogenicity                  | At least one bipoles has median EI > .3 | Bipole | 14 | 1.768 | .184 |
| Anatomical zone                   | 3 intrazones             | Patient   | 13 | 1.684         | .431   |
| Epileptic spasms                  | Absent or present as current seizure type | Patient-bipole | 14 | 4.552 | .033 |
| Anatomical zone * epileptic spasms| Patient-bipole           | Patient-bipole | 13 | 7.909 | .020 |

Abbreviations: EI, epileptogenicity index; EZ, epileptogenic zone; SEEG, stereo-electroencephalography.

**Figure 2** Functional connectivity according to epileptic spasms (ES) and anatomical zone. (A, B) Functional connectivity (\( h^2 \)) involving all intrazone connections. (A) Boxplot of \( h^2 \) values comparing absent (no ES) and present ES (ES) as a current seizure type. (B) Boxplot of \( h^2 \) values according to both ES and intrazones. (C, D) Functional connectivity (\( h^2 \)) involving all interzone connections. (C) Boxplot of \( h^2 \) values comparing absent (no ES) and present ES (ES) as a current seizure type. (D) Boxplot of \( h^2 \) values according to both ES and interzones. DC, distant cortex; DT, dominant tuber; NC, nearby cortex; OT, other tubers. \(^* p < .05.\)
examine the relationship between ES and $h^2$ according to each anatomical intrazone (Table 3, Figure 2B). Increased coupling within the tuber zone (.002) and DC zone ($p = .025$) was associated with ES, and a similar trend was observed in nearby cortex ($p = .061$).

### 3.3 Predictors of interzone FC

The factors associated with FC ($h^2$) were next investigated across all interzone connections (between dominant tuber, other tubers, nearby cortex, and distant cortex combinations; Table 4). The optimal multivariate model with the best fit was chosen according to BI-AI criteria and with acceptable conditional $R^2$ (.467), Hosmer–Lemeshow goodness-of-fit test ($p$-value = 1), and ICC (.066) and normal residuals.

Interictal FC ($h^2$) was independently associated with shorter distance between bipoles ($df = 20$, chi-squared = 69.3, $p < .001$), increased spike rate ($df = 20$, chi-squared = 67.9, $p < .001$), the presence of ES as a current seizure type ($df = 21$, chi-squared = 6.4, $p = .011$), and the anatomical interzone ($df = 16$, chi-squared = 34.1, $p < .001$; Figure 2C).

Similar to the intrazone analysis, we observed that FC was not associated with EZ organization or epileptogenicity.

The anatomical zone–ES interaction variable was significantly associated with $h^2$ ($df = 16$, chi-squared = 37.6, $p < .001$). We again performed post hoc analyses to investigate the relationship between ES and $h^2$ according to anatomical interzones (Table 3, Figure 2D). Increased coupling was associated with ES across all interzones with the exception of the nearby cortex connections with OT and DC.

### 4 DISCUSSION

This is the first study to examine FC with intracerebral EEG in patients with TSC. Our major findings are that (1) patients with ES have increased FC across a range of anatomical cortical networks, predominantly those involving tubers; and (2) epileptogenicity and EZ organization are not correlated with FC within or between tuber and non-tuber zones.

### 4.1 FC and ES

In our study, we identified that patients with ES displayed higher interictal FC in local and global cortical networks involving both tubers and normal-appearing cortex. Overall, these findings suggest widespread cortical synchrony is characteristic of patients with ES. The exact mechanisms underlying the generation of ES are poorly understood. Although ES are overrepresented in patients with TSC, they can occur in a heterogenous group of childhood epilepsies. Recent studies have supported the concept of a hyperconnected network being critical for the clinical manifestation of spasms in TSC and other pathologies.38

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**TABLE 3** Association between $h^2$ functional connectivity and epileptic spasms according to anatomical zones

| Anatomical zones                | Ratio | SE   | $p$  |
|---------------------------------|-------|------|------|
| Intrazone                       |       |      |      |
| Tuber                           | .679  | .118 | .002 |
| Nearby cortex                   | .720  | .127 | .062 |
| Distant cortex                  | .679  | .118 | .025 |
| Interzone                       |       |      |      |
| Dominant tuber–other tubers     | .5292 | .1133| .003 |
| Dominant tuber–nearby cortex    | .5561 | .1151| .005 |
| Dominant tuber–distant cortex   | .5911 | .1201| .010 |
| Other tubers–nearby cortex      | .7678 | .1483| .171 |
| Other tubers–distant cortex     | .6144 | .1181| .011 |
| Nearby cortex–distant cortex    | .7438 | .1425| .122 |

*Note*: Anatomical zones are schematically displayed in Figure 1.

**TABLE 4** Multivariate predictors of interzone functional connectivity

| Variable                    | Variable information | Level | $df$ | $\chi^2$ | $p$  |
|-----------------------------|----------------------|-------|------|----------|------|
| Age at SEEG                 | Years                | Patient | 1    | .220     | .639 |
| Number of type C tubers     | Number               | Patient | 1    | 1.810    | .179 |
| Number of total tubers      | Number               | Patient | 1    | .328     | .567 |
| Distance between bipoles    | Euclidean distance   | Bipole | 1    | 69.315   | <.001|
| Spike rate                  | Mean between bipoles | Bipole | 1    | 67.868   | <.001|
| EZ organization             | Focal vs. complex    | Patient | 1    | .116     | .148 |
| Epileptogenicity            | At least one bipolar has median EI > .3 | Bipole | 1    | 2.089    | .733 |
| Anatomical zone             | 6 interzones         | Bipole | 5    | 34.088   | <.001|
| Epileptic spasms            | Absent or present as current seizure type | Patient | 1    | 6.423    | .011 |
| Anatomical zone * epileptic spasms | Patient-bipole | 5    | 37.630 | <.001 |

*Abbreviations*: EI, epileptogenicity index; EZ, epileptogenic zone; SEEG, stereo-electroencephalography.
By utilizing SEEG data with high spatial resolution, we were able to interrogate specific tuber and nontuber networks. Overall, FC was broadly increased across a variety of anatomically defined networks in patients with ES. Post hoc analyses showed that interzone networks involving the dominant tuber in patients with ES displayed the highest coupling; in contrast, zones involving nearby normal-appearing cortex tended to yield nonsignificant trends. Although this may support the dominant tuber having a more crucial role in these ES-generating networks, we also observed significantly increased FC in networks involving only normal-appearing cortex (ie, distant cortex intrazone FC).

These findings are in keeping with a recent lesion-mapping study of 123 children with TSC, which found that global cortical and subcortical network alteration was present in patients with ES. The authors utilized a statistical mediation analysis and determined that increased tuber burden led to strong negative connectivity with globi pallidi, which predicted ES. In their study, there was no single anatomical brain region that was affected by tubers in all cases with ES. However, the study did not account for the underlying epileptogenicity of tubers. In contrast, we found that the total tuber number, including the number of highly epileptogenic type C tubers, was not associated with FC on multivariate analysis.

Extratuber abnormalities, particularly those involving white matter tracts, may be important for the increased FC observed in patients with ES. It is well recognized that patients with TSC have diffuse brain abnormalities extending beyond anatomical tubers. Immunohistological studies have described disorganized axonal projections with increased axonal connectivity in perituberal tissue. Structural diffusion tractography studies have identified connectivity changes in normal-appearing white matter. Also white matter tractography abnormalities in TSC patients have been associated with autism spectrum disorder, and machine-learning models that analyze structural MRI abnormalities can accurately predict major neurocognitive morbidity.

4.2 EZ organization and FC

Our second objective was to examine the relationship between epileptogenic regions and FC. We found that the degree of coupling between tubers and nearby cortex is not dependent upon the spatial pattern of EZ organization (at a patient level) nor the epileptogenicity of sampled structures (at a connection level). This finding is in contrast to our original hypothesis, that patients with a focal EZ confined to a dominant tuber would exhibit a unique and restricted pattern of connectivity.

Our results may reflect the interictal FC methodology that was utilized. In this study, spike rates were highly correlated with the nonlinear measure of connectivity. The irritative zone is typically defined by the presence of interictal discharges and generally extends beyond the EZ. In the same cohort studied in this analysis, we have previously reported that continuous interictal epileptiform discharges often propagated into both nearby and distant cortical structures. Therefore, it is possible that increased $h^2$ is reflecting the pattern of the irritative zone rather than the EZ organization. Another explanation for this finding is the methodology of defining a connection between two regions as epileptogenic if at least one region, but not necessarily both, had a high EI. This may have limited the ability to find a statistical association between bipole level epileptogenicity and FC.

4.3 ES and epileptogenic tubers

The relationship between epileptogenic tubers and ES is not straightforward. First, it is well established that seizures can arise from a single epileptogenic tuber and in some patients (including some in this cohort) such seizures can clinically manifest as ES and seizure freedom can occasionally be achieved with tuberecctomy alone. However, this is not the rule, and in this study ES were equally seen across both focal and complex EZ organizations. Our findings, together with recent studies, support the concept that ES is associated with a network characterized by increased cortical synchrony. This network dynamic may be largely driven by widespread connectivity and anatomical alterations, rather than tuber epileptogenicity. We hypothesize that this could be explained by the presence of distinct, pathological networks in patients with TSC; for example, an EZ network that generates seizures and the diffuse hypersynchronous network that enables some seizures to manifest as ES.

However, the exact causal relationship between ES and increase FC is not understood. Is cortical hypersynchrony required for the manifestation of ES or do ES help drive increased coupling? In support of the former hypothesis is a recent scalp EEG-based FC study showing that increased connectivity can be observed before the onset of spasms. Additionally, a separate study reported that a reduction in FC after adrenocorticotropic hormone or vigabatrin treatment predicted suppression of spasms, although it did not predict seizure freedom from other seizure types. Overall, the role of epileptogenic tubers and ES in generating or maintaining this hypersynchronous network remains uncertain.
4.4 Limitations

This was a retrospective study, with only a small sample of patients with ES. The small study cohort is a major limitation of this analysis. There was no standardized, long-term quantification of neurocognitive and development outcomes after surgery and resolution of spasms. Also, only one third of tubers were sampled in each patient. Additionally, this study would have been strengthened by scalp EEG-derived FC measures recorded before, during, and after the SEEG recording to help validate noninvasive biomarkers. SEEG sampling of subcortical structures was not routine across centers; however, these data would have allowed a more complete connectivity analysis in light of more recent literature. Correlating FC within the boundaries of resection and postoperative seizure outcome was not performed and could be examined in future research. Standardizing the interictal period relative to arousal from sleep would have additionally improved homogeneity of epochs. Finally, incorporating FC directionality would have expanded the ability to interrogate the role of dominant tubers in the zones with increased FC.

4.5 Future research

In TSC, interictal discharges can precede the onset of clinical seizures, and preliminary evidence suggests that prophylactic treatment before the onset of ES can improve neurocognitive outcomes. A prospective, multicenter randomized–controlled trial (EPISTOP) has examined clinical and electrographic biomarkers of epilepsy and development delay in infants with TSC. Early life quantitative EEG characteristics correlated with autism spectrum disorder risk at 24 months. Additionally, in patients with subclinical epileptiform discharges, vigabatrin reduces the risk of clinical seizures, including ES. A second similar trial is currently underway (PREVENT, NCT02849457).

5 CONCLUSIONS

Our study has found that widespread cortical synchrony is associated with the presentation of ES in patients with TSC. Future studies, including active prospective projects, should focus on the identification of reliable noninvasive biomarkers of increased FC that can be analyzed at an individual patient level. These FC biomarkers may provide a more sensitive and earlier indicator of the development of ES, epilepsy, and developmental encephalopathy and therefore optimize presymptomatic treatment paradigms.
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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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