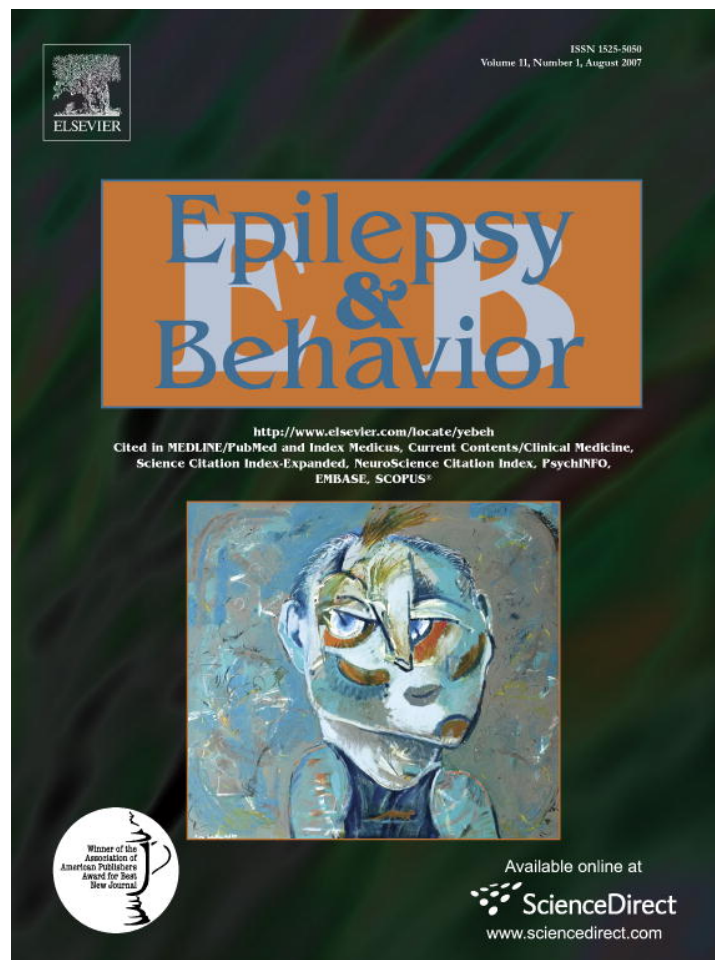


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## Case Report

## Local epileptogenic networks in tuberous sclerosis complex: A case review

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**Abstract**

**Objective.** Cortical tubers are a pathognomonic finding in some patients with tuberous sclerosis complex (TSC), and are believed to be epileptogenic foci. Surgery is an effective option in selected patients with TSC who are refractory to medical therapy. This article describes three patients with TSC who underwent three-stage epilepsy surgery at our center, with the intention of examining local electrophysiological changes after each stage of the procedure.

**Methods.** Magnetic resonance images were obtained after initial implantation of electrodes and after resection and electrode reimplantation. These images were co-registered and overlaid. The intracranial grids were overlaid in a similar procedure and manually traced, and then added to the volumetric image. Mean spike counts were obtained for each patient and expressed in spikes per minute. Statistical analysis was performed on spike counts prior to and after resection.

**Results.** All three patients displayed intense spiking in the regions around the dominant epileptogenic tuber. On tuber removal, spike counts diminished significantly. In each case, new areas of spiking emerged in regions remote from the tuber periphery after tuber resection, with the emergence of secondary ictal onset zones in the resection margin.

**Conclusion.** This retrospective study highlights some common electrophysiological features among the patients examined. The observed epileptogenic activity and regions of ictal onset suggest that it may be the region of brain tissue surrounding the tuber that is responsible for the majority of epileptogenic activity in these patients.

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**Keywords:** Tuberous sclerosis; Networks; Three-stage surgery**1. Introduction**

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystemic disorder that causes significant neurological sequelae. Up to 90% of affected individuals may develop medically refractory epilepsy and developmental impairments, including mental retardation and autism [1]. Cortical tubers, which are foci of dysplastic neuronal tissue and gliosis, are a pathognomonic finding in some patients with TSC. These tubers are believed to be epileptogenic foci. Antiepileptic drugs (AEDs) are often used in polytherapy to obtain seizure control. Effective control of seizures is critical in TSC, as there exists a strong association

between early seizure onset, refractory epilepsy, and poor developmental outcome [2–4]. The widespread nature of cortical dysfunction and epileptogenicity in those with TSC is evidenced by the combinations of partial and symptomatic generalized seizures (e.g., infantile spasms), other structural central nervous system abnormalities (e.g., subependymal nodules, white matter heterotopia), mental retardation, autistic spectrum symptoms, and poor surgical outcome [5]. During infantile spasms in patients with TSC, electrocorticography reveals rapid and diffuse neocortical propagation of epileptic activity [6].

Surgery is an effective option in selected patients with TSC. As current imaging and electrographic techniques allow for better localization and characterization of epileptogenic networks in TSC, surgical outcomes have improved. However, multifocal cortical epileptogenic

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abnormalities occur in these patients, as demonstrated by intracranial electroencephalography findings. Furthermore, latent epileptogenic networks that are suppressed by the prominent epileptogenic activity of a tuber are likely present in most patients with TSC and may explain seizure recurrence following tuber resection. These phenomena become more problematic as intracranial electroencephalography has significant spatial limitations. To investigate and improve detection of these networks and surgical outcomes, our group has developed a novel surgical strategy that involves a three-stage invasive approach. Patients are implanted with subdural electrodes, with subsequent resection of the ictal onset zone based on analysis of the intracranial electroencephalographic data. Following this initial stage, the resection zone and surrounding areas are immediately reimplanted with subdural electrodes to identify any residual seizure foci. Some patients have a second resection if new epileptogenic areas are identified during the second stage.

We examined a case series of three patients with TSC who underwent three-stage invasive monitoring and surgery at our center. These patients were previously reported by Weiner et al. as part of a study cohort examining surgical outcomes of three-stage monitoring [7]. However, the objective of this study is to provide a superficial examination of dynamic electrophysiological changes in local cortical networks resulting from this surgical procedure, with the intention of performing a more in-depth prospective study in the future. We examined interictal and ictal discharges on co-registered magnetic resonance images in patients after each stage to better define local epileptogenic networks within the cortex immediately surrounding the resection zone. This investigation focused exclusively on the electrographic features of grid electrodes because these provide a contiguous sample of cortical activity, and are not subject to the same degree of selection bias as strip electrodes.

## 2. Methods

Three children with TSC who underwent three-stage resective surgery at NYU Comprehensive Epilepsy Center by a single pediatric neurosurgeon (H.L.W.) were studied. The patients were selected on the basis of: occurrence of clinical seizures prior to and after the initial tuber resection, adequate grid electrode coverage of the ictal onset zone, and the presence of high-quality MRI studies (pre- and postop) allowing performance of the co-registration procedure. Images underwent a 12-point three-dimensional co-registration process using the FMRIB Software Library (FSL) toolbox [8], and were exported to MRICro [9], where a skull stripping algorithm was performed. Volumetric magnetic resonance image files were created with surface views of the intracranial electrodes. The results of surgery in some of these patients have been reported previously [7].

Intracranial electroencephalographic data were collected from each patient using a Nicolet Biomedical EEG system, digitized at a sampling rate of 400 Hz. Based on visual analysis of the intracranial electroencephalogram (iEEG), seizure onset and irritative zones within each stage were identified. The sagittal magnetic resonance images obtained above were then exported into the GNU Image Manipulation Program (GIMP). Electrodes comprising ictal and interictal activity were highlighted and overlaid on the image using a multilayering process. The intracranial grids

were overlaid in a similar procedure and manually traced, and then added to the volumetric image. Approximate resection zones were also traced based on available neurosurgical data and visual analysis of the reconstructed volumetric MRI scans. If possible, the volumetric MRI scan was examined for the presence of visually identified tubers, and these were traced as well.

Based on availability of archived iEEG data, five random 10-min interictal epochs of iEEG on each patient were identified for spike counts on separate days of recording. Because of the arbitrary nature of archived data, patient characteristics such as activity level and state of vigilance were not examined. Interictal data that were obtained 3 h prior to or after a seizure were excluded. Mean spike counts were obtained on each set of five samples, and expressed in spikes per minute. Statistical analysis was performed on spike counts prior to and after resection with the Wilcoxon signed-rank test, using zero values to create data sets of identical size.

## 3. Results

There were no perioperative complications. Per the last clinical visit notes or telephone follow-ups (at least 1 year after procedure), all patients are seizure-free with clinical improvement in cognition and developmental milestones.

### 3.1. Patient 1

This 2-year-old boy was diagnosed with TSC at 6 months, after his first seizure, which was characterized by tonic stiffening of his extremities with eye deviation to the left. He developed spasm-like events for the next month, and failed trials of phenobarbital and clobazam. Vigabatrin was initiated, which resulted in the cessation of seizures for 6 months. At age 14 months, he experienced an hour-long tonic seizure, followed by several tonic seizures over the next several weeks. Oxcarbazepine was added to vigabatrin, without improvement. Prior to surgery, he experienced prolonged tonic seizures once every 1–3 months, with 3–10 brief staring episodes daily. He had experienced language regression and increased impulsivity and aggression since the onset of seizures. Seizures captured by scalp video/EEG (VEEG) monitoring revealed electrographic onsets in the right centroparietal regions. Interictal discharges consisted of generalized spike-wave complexes with bilateral frontal predominance. MRI revealed scattered parenchymal hamartomas, consistent with tubers.

At the initial stage of the surgery, he underwent a right craniotomy with a 30-contact grid placement, with four additional strips. During monitoring, he experienced multiple seizures, consisting of multiple subclinical episodes and seizures characterized by whole-body jerks with bilateral arm extension. Based on visual analysis of ictal onsets, a large right frontoparietal tuber was resected, together with tissue in surrounding cortex in which electrodes showed ictal onset. He was reimplanted with a 32-contact grid over the resection (Fig. 1). On Day 5 following reimplantation, the patient experienced a 4-h episode of partial status epilepticus, characterized by intermittent turning of the eyes to the left and left arm clonic movements. This

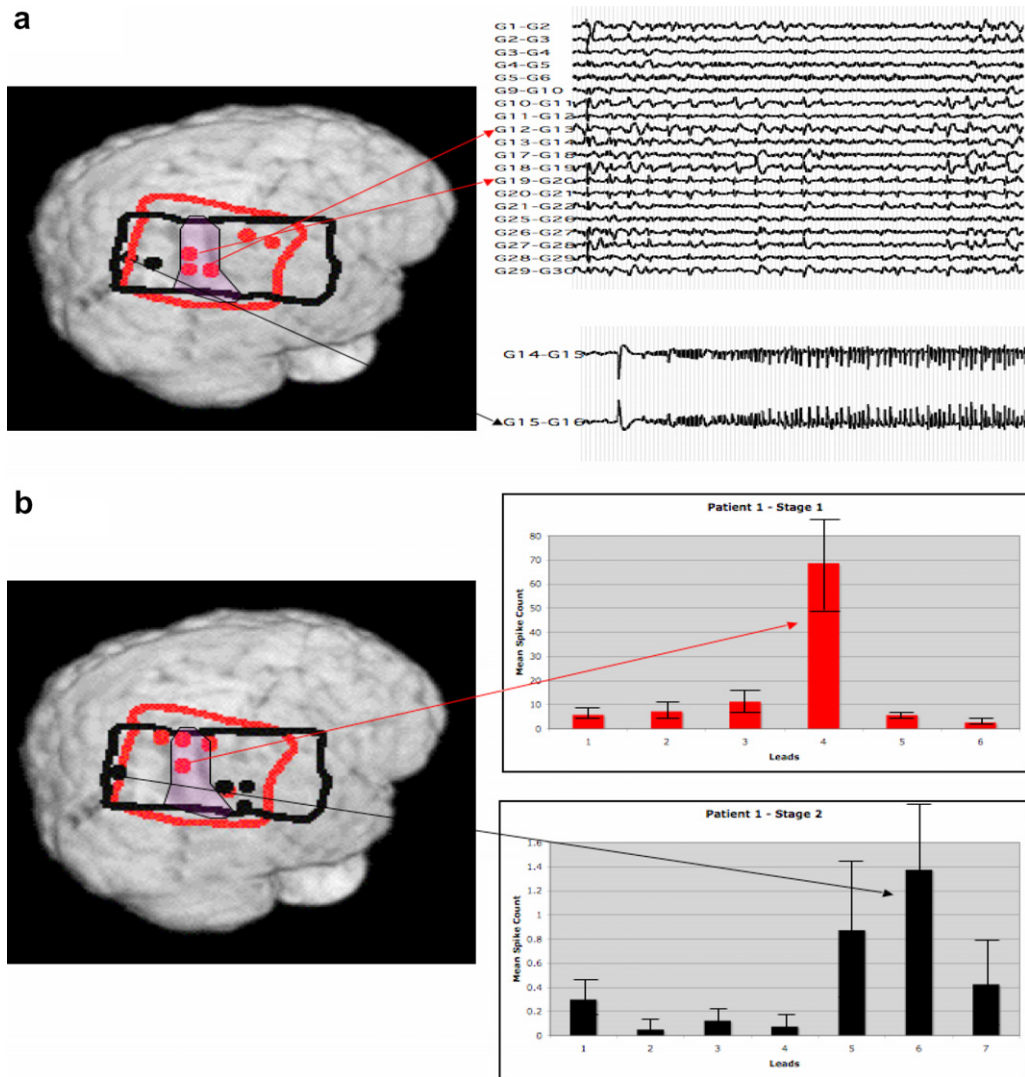


Fig. 1. Ictal (a) and interictal (b) maps for Patient 1. Grid outlines were traced after stage 1 (red) and stage 2 (black) implantations. Active electrodes from stage 1 (red) show ictal onsets in the posterior margin of the tuber (approximate tuber outline designated by shaded area), with a related region of ictal onset anterior to the tuber. Interictal spikes were noted over the anterior and superior edges of the tuber. Marked diminishment of spike counts was noted after resection of the tuber. Electrodes with counts less than 0.2 spike/min are not represented in the figure. Please note the difference in Y axes in spike counts between stages 1 and 2. Error bars represent one standard deviation from the mean. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

appeared to arise from the posterior resection margin. In addition, frequent interictal activity around the resection margin was noted, as was a new area of interictal activity in the frontopolar region in the area of a known tuber. Two days later, the area of seizure onset and interictal activity was resected around the posterior margin of the previous resection.

Examination of interictal data (Fig. 1b) reveals an extremely active spiking region (~70 spikes/min) in the posterior margin of the outlined tuber, which was involved in the onset region of status epilepticus following resection of the tuber. After tuber resection, however, the overall interictal spike counts decreased dramatically ( $P < 0.0001$ ), with the maximum spiking region around 14 spikes/10 min, which is also in the posterior margin of the resected tuber.

### 3.2. Patient 2

This 13-month-old boy was diagnosed with infantile spasms at age 5 months. MRI revealed multiple tubers, with the largest in the right parietal lobe. He was initially started on adrenocorticotrophic hormone (ACTH), which did not have an effect. Vigabatrin resulted in seizure freedom for 2 months. Infantile spasms recurred, and did not respond to trials of oxcarbazepine, lamotrigine, or zonisamide. He also developed tonic seizures characterized by truncal flexion to the right. With recurrent seizures, his development failed to progress and then he regressed. Scalp VEEG monitoring data from typical seizure episodes revealed multifocal spikes with generalized paroxysmal fast activity. Interictally, frequent bilateral central, temporal,

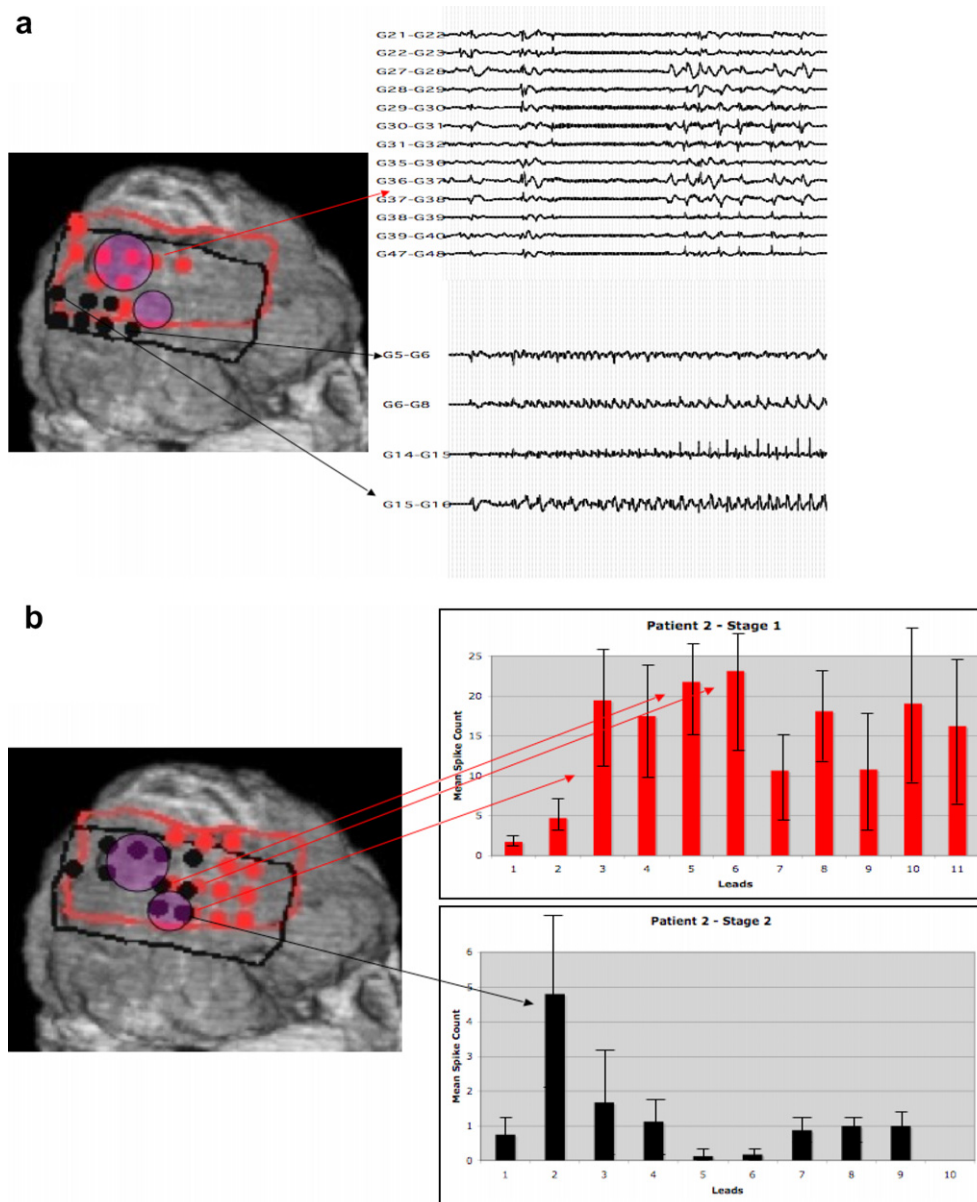


Fig. 2. Ictal onsets (a) and interictal spiking rates (b) in Patient 2. The major region of ictal onset (electrode 36) and interictal activity appeared to cluster in tissue over the anterior edge of the tubers (designated by shaded circles). As with Patient 1, a significant decrease in spiking rates is seen after removal of the tuber and adjacent epileptogenic cortex. Please note the difference in Y axes in spike counts between stages 1 and 2. Error bars represent one standard deviation from the mean.

and parietal spikes were observed, with predominance in the right posterior quadrant.

Based on MRI and VEEG monitoring data, he underwent a right craniotomy with a 64-contact grid placement, centered on the right parietal tuber. Three additional strip electrodes were placed over the right hemisphere, and six strip electrodes were placed over the left hemisphere through a burr hole. During monitoring, he experienced several typical seizures, with tonic elevation of the arms and flexion of the trunk. The EEG revealed widespread ictal onsets with a diffuse electrodecrement with superimposed fast activity, maximal around the large tuber (Fig. 2a). After 1 week of monitoring, he underwent resec-

tion of the tuber and surrounding cortex with recorded ictal activity, followed by reimplantation of a 32-contact grid over the resection. The six strip electrodes over the left hemisphere were maintained from the initial stage. One day after reimplantation, he had multiple seizures, both subclinical and with behavioral arrests (a new semiology), with discrete onsets around the postero-inferior edge of the resection margin, with immediate spread to the antero-inferior edge of the resection. In addition, significant interictal activity was noted in these regions. Also, broadly distributed spike and polyspike activity was seen in the strip electrodes over both hemispheres. After 8 days of monitoring, the tissue underlying the electrodes with ictal activity was

resected, and an ipsilateral frontal topectomy was performed over strip electrodes recording the polyspike activity.

Interictal spikes revealed a significant number of active electrodes around the periphery of the primary tuber (Fig. 2b), with spiking rates between 20 and 25 spikes/min. After resection of the tuber, a significant diminishment of spiking activity was seen, similar to Patient 1 ( $P < 0.0001$ ). This area correlated with the ictal onset zone following resection.

### 3.3. Patient 3

A 3-year-old girl was diagnosed with TSC at age 6 months, when she developed refractory infantile spasms and later partial seizures. She failed trials of multiple AEDs, including ACTH, vigabatrin, levetiracetam, zonisamide, clonazepam, lamotrigine, and topiramate, as well as the ketogenic diet. She continued to have approximately four complex partial seizures per day, characterized by behavioral arrest with staring and blinking. After seizure onset, she experienced multiple developmental delays affecting motor skills and language. Scalp VEEG data from multiple episodes of her usual seizures revealed 4.5-Hz spike-wave activity originating from the left frontotemporal region. MRI revealed multiple areas of cortical white matter abnormalities and bilateral subependymal giant cell astrocytomas in the foramina of Monroe.

She underwent a left craniotomy with placement of a 64-contact grid over the hemisphere and five strip electrodes surrounding the grid. During monitoring, she experienced 12 seizures characterized by eye blinking and behavioral arrest, with electrographic onsets in the left inferior parietal region (corresponding with a known tuber) and an additional frontal region with no known pathology (Fig. 3). Frequent interictal spike and wave activity was noted in the corresponding regions. After 5 days of monitoring, she underwent resection of the parietal tuber and surrounding regions of ictal onset, with reimplantation of a 64-contact grid, with one 4-contact strip electrode located in the frontal region, over an identified tuber. After 3 days of monitoring, she experienced her usual episodes of eye blinking, which showed electrographic onsets around the posterior–inferior margin of the resection. Also, frequent interictal activity was seen around the resection margin. After three additional days of monitoring, the patient underwent additional resection of the seizure focus along the posterior border of the previous resection margin.

A distributed region of interictal spiking was observed prior to resection of the main tuber, with active areas in a small region anterior to the tuber and in the frontal lobe. Interestingly, the frontal lobe spikes were most robust, with rates at approximately 4–5 spikes/min. After resection of the main tuber in the superior temporal region, however, spiking activity was markedly diminished in the regions immediately anterior to the grid and in frontal areas located further anteriorly as well ( $P < 0.0001$ ). However,

the region of grid coverage does not extend as anteriorly in the second stage as it does in the first.

## 4. Discussion

Our data appear to reveal certain common electrophysiological patterns in the three-stage invasive approach among these patients, before and after the first resection. Patient 1 displayed a region around a single electrode with very intense interictal spiking activity prior to resection (Fig. 1). This region was also involved in the ictal onset prior to the initial resection, and tissue adjacent to this area (which was resected) appeared to become the onset zone for the episode of status epilepticus the patient experienced after the resection. Interestingly, the regions of spike activity and ictal onsets after the resection were in regions that were not active prior to resection of the tuber. However, the regions of interictal spiking noted around the tuber significantly diminished on resection of the tuber. Patient 2 also displayed regions of significant interictal spiking around the edges of the tubers (Fig. 2), areas that also appeared to contribute to ictal onsets. As with Patient 1, resection of the tubers resulted in diminished spike counts; however, these spikes were identified in areas that were previously not interictally active. Patient 3 also displayed this phenomenon; however, the areas of diminished spiking were remote from the resected tuber. Ictal onsets in Patient 3 originated from the resection margin, which was consistent with Patients 1 and 2.

In all patients, we found evidence of recurrent epileptogenic activity around the area of primary epilepsy tissue resection, as well as in areas remote from the resection. The ictal onset zones after reimplantation showed localization around the initial resection margin in all three patients. This could indicate abnormal neuronal epileptogenicity secondary to local injury effects, or residual epileptogenic tissue that was incompletely resected after the initial stage. Interictal EEG data after the first resection revealed continued interictal spiking around the resection margin. As these spikes were present only in discrete regions around the resection, a general injury effect is unlikely to fully account for this observation. More likely, additional epileptogenic cortex remained after the first invasive recording and resection.

New regions of interictal spiking emerged in all three patients remote from the area of resection. This likely indicates a disinhibitory effect of local epileptogenic networks, where new areas displayed activation after removal of the initial ictal focus. Also, these areas all showed markedly lower spiking rates when compared with the original spiking activity around the ictal onset zones prior to resection. This could be attributed to the creation of a surrounding inhibitory zone around the intense interictal spiking activity of the ictal onset zone, where highly epileptogenic tissue in or around the tuber suppresses surrounding areas via the activation of inhibitory interneurons [10]. Large-scale epileptogenic networks affecting the activity of distal brain

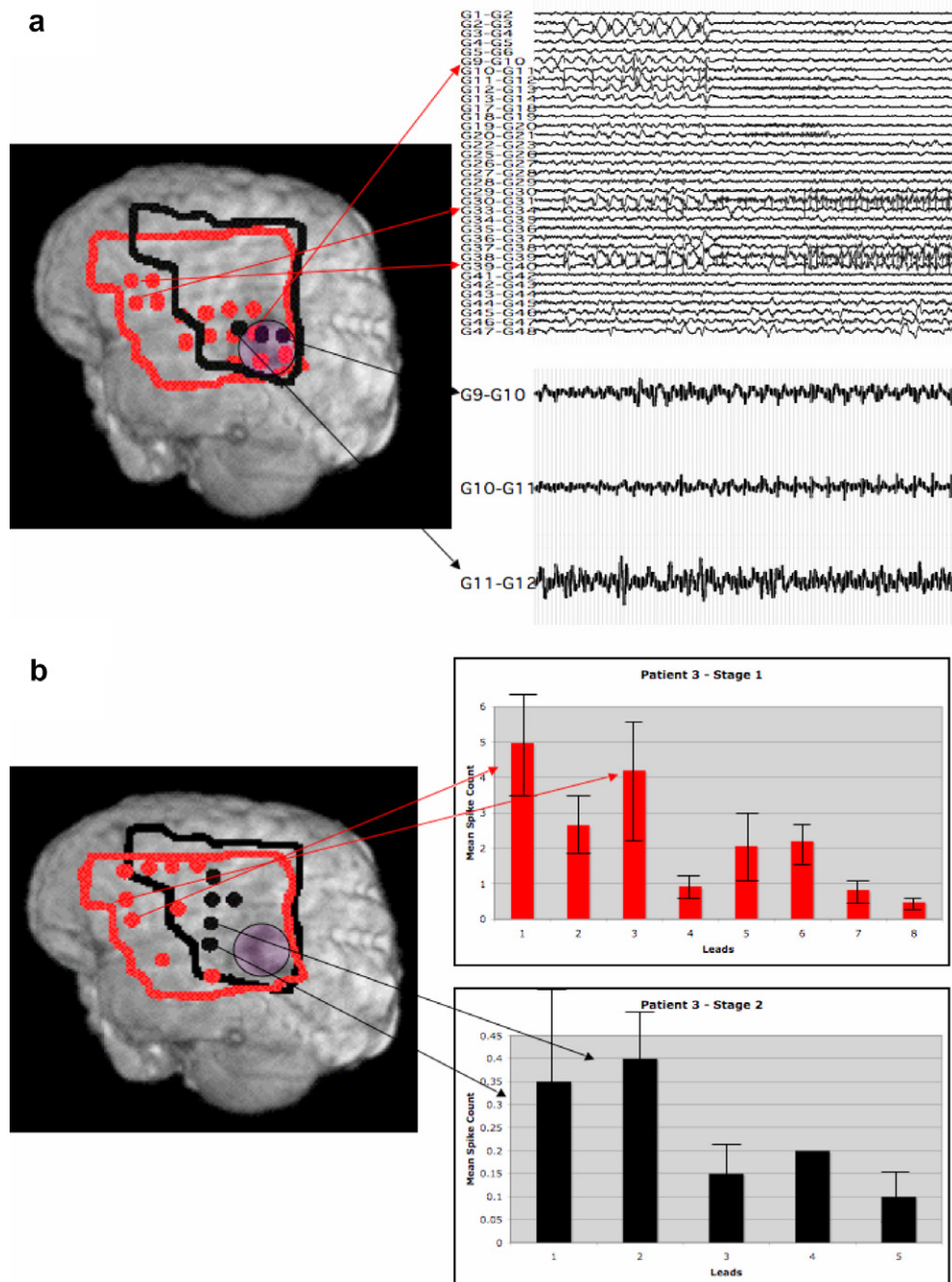


Fig. 3. Ictal onsets (a) and interictal spiking rates (b) in Patient 3. Widespread ictal onsets were noted anterior to the dominant tuber (shaded circle), with the most robust onsets seen in the frontal region. This was corroborated by the interictal spiking rates, which were maximal in the same frontal electrodes. After tuber resection, ictal onsets appeared to initiate from the anterior edge of the resection margin. After tuber resection, areas of frontal spiking seen in the initial stage of monitoring do not appear to be present, although the extent of grid coverage does not extend as far frontally in the second stage as compared with the first. Please note the difference in Y axes in spike counts between stages 1 and 2. Error bars represent one standard deviation from the mean.

structures and associated surround inhibition [10,11] have been demonstrated in ictal foci, and appear to be demonstrated in Patient 3, with attenuation of spiking in frontal regions after tuber resection in the posterior temporal area. Alternatively, the presence of interictal spikes in the area of the resection margin could be inhibitory in themselves [12,13], suppressing the creation of new ictal onset zones. In such a case, it could be argued that removal of all spik-

ing cortex could remove natural inhibitory mechanisms. Our electrophysiological observations are consistent with the hypothesis that a combination of local neuronal epileptogenicity surrounding a tuber creates a local network that is dramatically disrupted on tuber resection, which in turn causes a loss of this surround inhibition in neighboring regions, resulting in the appearance of interictal discharges remote from the tuber resection margin.

A potential confounding factor is the alteration of spiking rates due to the withdrawal of AEDs during monitoring, to provoke seizures. However, any AED changes would likely impact the entirety of the recording area proportionally, and would preserve the comparative spiking rates of each region. This study is also limited by the lack of an accurate trace of the resection zone, as the outlines were traced based on the resection documented by neurosurgical records and figures as well as postop MRI, in consultation with the operating neurosurgeon. An exact spatial trace is imprecise due to the shifting of surrounding brain tissue around resection margins, where there is often a “filling-in” effect of the space created by resection around surrounding brain. Therefore, as the exact resection margin is unknown, the edge effects noted above are approximate as well. Based on these results, it appears that a prospective study is indicated, with the exact location of surgical resection determined intraoperatively. Patient characteristics that would be critical to prospectively examine when performing spike counts include state of arousal and daily serum AED levels. In addition, histopathological analysis of resections is planned as well, with particular attention paid to the region of brain around tuber.

The investigation described here highlights an emerging concept in the area of ictogenesis in TSC, that is, the role of brain tissue surrounding the tuber. This phenomenon has been demonstrated using [<sup>18</sup>F]fluoro-2-deoxyglucose positron emission tomography (FDG-PET) and MRI apparent diffusion coefficients (ADCs) by Chandra et al. [14], where areas of larger FDG-PET hypometabolism and higher ADC values identified epileptogenic tubers and surrounding dysplastic cortical tissue. This was further corroborated by Juhasz et al. [15], who demonstrated high degrees of epileptogenic potential arising from cortical tissues adjacent to tubers identified by  $\alpha$ -[<sup>11</sup>C]methyl-L-tryptophan (AMT) PET, which selectively identifies epileptogenic tubers. Additionally, white matter abnormalities surrounding tubers have been demonstrated using diffusion tensor imaging (DTI) by Karadag et al. [16]. The aforementioned studies reveal an extensive interaction between the tuber and surrounding tissue, which is often structurally abnormal as well. This interaction appears to disrupt normal tissue metab-

olism and enhance epileptogenicity. These findings suggest that wider resections may be necessary around the dominant tuber to fully remove the ictal onset zone.

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