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Reproducibility of Interictal Spike Propagation in Children with Refractory Epilepsy

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Abstract

Objective: Interictal spikes are a characteristic feature of invasive electroencephalography (EEG) recordings in children with refractory epilepsy. Spikes frequently co-occur across multiple brain regions with discernable latencies, suggesting that spikes can propagate through distributed neural networks. The purpose of this study was to examine the long-term reproducibility of spike propagation patterns over hours to days of interictal recording.

Methods: Twelve children (mean age = 13.1 years) were retrospectively studied. An average of 47.2 ± 40.1 hours of interictal EEG were examined per patient (range = 17.5 – 166.5 hours). Interictal recordings were divided into 30-minute segments. Networks were extracted based on the frequency of spike co-activation between pairs of electrodes. For each 30-minute segment, electrodes were assigned a 'Degree Preference (DP)' based on the tendency to appear upstream or downstream within propagation sequences. The consistency of DPs across segments ('DP-Stability') was quantified using the Spearman rank correlation.

Results: Regions exhibited highly stable preferences to appear upstream, intermediate, or downstream in spike propagation sequences. Across networks, the average DP-Stability was 0.88 ± 0.07 , indicating that propagation patterns observed in 30-minute segments were representative of the patterns observed in the full interictal window. At the group level, regions involved in seizure generation appeared more upstream in spike propagation sequences.

Keywords

Interictal spike; propagation; network; epilepsy surgery; invasive EEG

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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1. Introduction

Interictal spikes are electrographic paroxysms arising when local populations of cortical neurons become abnormally synchronized.¹ In addition to local spike activity, researchers have long recognized the tendency for regions to co-activate during spike discharges with millisecond-scale latencies,^{2,3} suggesting that spikes can propagate across the cortex.^{4–16} Spike propagation is thought to reflect the transient activation of neural networks involved in the generation and transmission of epileptiform discharges.¹⁷ Mapping spike propagation appears to be clinically relevant, as studies find that regions appearing early (i.e., ‘upstream’) in propagation sequences preferentially localize to the SOZ^{6,7,9} and must be excised to achieve seizure-freedom.¹⁸ Repeated co-activation of regions during spike discharges has also been theorized as a mechanism of pathological network formation and epileptogenesis.^{1,19,20} Given that the interictal state accounts for the vast majority of EEG recordings, improved understanding of spike propagation and network dynamics is key to advancing disease models of epilepsy and increasing the yield of the pre-surgical evaluation.

An important issue when characterizing spike propagation is establishing the reproducibility of network co-activation patterns over time.^{5,21} In this context, ‘co-activation patterns’ refer to the millisecond-scale latency differences observed between spikes at distinct brain regions, and ‘reproducibility’ refers to the spatiotemporal consistency of co-activation patterns over many propagation discharges. Most studies rest on the assumption that co-activation patterns observed in relatively brief EEG segments (e.g., <60 minutes) are representative of the long-term behavior of spike networks, but the well-documented spatial and temporal variability of spikes^{22–25} demands a skeptical evaluation of this assumption. To advance our understanding of network-level pathophysiology, we investigated the long-term reproducibility of interictal spike propagation in 12 children who underwent invasive monitoring with subdural electrodes. We tested the hypothesis that spike co-activation patterns are reproducible over hours to days of interictal recording.

2. Materials and Methods

2.1. Patient selection and intracranial EEG recording.

This study was approved by the Children’s Hospital of Philadelphia (CHOP) Institutional Review Board. Twelve patients who underwent long-term invasive EEG monitoring were retrospectively studied. Patients were selected from a larger institutional database on the basis of: (1) availability of seizure markings; (2) schematic electrode maps; (3) frequent interictal spiking documented in the EEG report; (4) at least two years of post-surgical follow-up; and (5) at least 16 hours of interictal EEG data for analysis (see Section 2.3: ‘Interictal segment selection’). Subdural electrodes manufactured by Adtech Medical Instruments Corporations (Racine, WI) were implanted (inter-electrode distance = 10 mm, exposure = 2.3 mm). Recordings were acquired at 200 Hz using a Telefactor Beehive 32–128 channel Cable Telemetry Encoder (CTE) digital synchronized video-EEG recording system (Astro Med Corp, West Warwick, RI). Online voltages were referenced to an electrode distant from the suspected epileptogenic zone. EEG data were subjected to an online 60 Hz notch filter. No offline filters or post-processing steps were applied.

All clinical decisions were made without influence from this study. Review of medical charts provided clinical information including seizure history, histopathology, and implant location. Post-surgical seizure outcome was assessed at least 24 months after surgery using the modified Engel scale.²⁶ Study procedures were conducted in accordance with the Declaration of Helsinki.

2.2. EEG inspection.

All uncorrupted EEG files were independently inspected by two experienced pediatric epileptologists. Periods of excessive EEG artifact (e.g., malfunctioning grid) were marked, and electrodes with persistent artifact were excluded to reduce data contamination. The earliest electrographic change (EEC) and onset electrodes were marked for each seizure. Disagreement between reviewers was resolved by discussion. Ten minutes of peri-ictal activity flanking each seizure were marked for rejection. The set of electrodes constituting the seizure network ('SZN'), which encompassed the SOZ and regions of consistent early ictal spread, was determined by expert review before conducting the interictal analyses.

2.3. Interictal segment selection.

For each patient, the longest window of interictal EEG activity without an interruption 2 hours in duration (e.g., peri-ictal epochs, file breaks, and artifact) was selected for analysis (Fig. 1A). Factors such as time-of-day, sleep-wake state, and anti-epileptic drug regimen were not considered when selecting the interictal window. The window was divided into non-overlapping 30-minute segments (Fig. 1A), and segments containing 3 minutes of rejected activity were discarded. The final dataset for each patient thus constituted a set of 30-minute segments spanning the longest available near-continuous window of interictal activity. The 30-minute segment duration was chosen to provide a sizeable number of spikes in each segment while remaining sensitive to changes unfolding over tens of minutes to hours.

2.4. Interictal spike detection.

Each 30-minute segment was submitted to an automated spike detection algorithm, which has been previously validated against human markings.²⁷ The mimetic-based detector identifies spikes based on a combination of amplitude and spectral characteristics. Detection parameters (e.g., amplitude threshold) were "tuned" for each patient as described in the original publication²⁷ using representative 5-minute interictal clips. Each spike was assigned an event time corresponding to the peak amplitude. In order to focus analyses on electrodes capturing frequent spikes, a non-parametric spike frequency threshold was applied (Supplemental Fig. S1). Electrodes with an observed spike frequency (spikes/min) exceeding 1.5 standard deviations above the mean of a surrogate distribution (iterations = 1,000) were analyzed.

2.5. Spike co-activation matrix.

Spike co-activation referred to the detection of spikes at distinct electrodes with brief inter-spike latencies (Fig. 1B).^{28,29} Consistent with previous studies, co-activation was defined as peak times separated by <150 ms.^{5,20,21} Spike co-activation rates were encoded in an

electrode-by-electrode matrix (C). For each electrode i , the co-activation rate between electrodes i and j ($C_{i,j}$) was given as: $C_{i,j} = (s_i | s_j) / s_i$, where $(s_i | s_j)$ was the number of spikes at electrode i with a co-activation spike at electrode j , and s_i was the total number of spikes recorded at i . Thus, $C_{i,j}$ represented the fraction of co-activations between electrodes i and j , normalized by the total spikes at electrode i (ranging from 0 to 1). Performing this calculation over all electrode pairs yielded the co-activation matrix, C (Fig. 1C). Note that C was non-symmetric, in that $C_{i,j}$ and $C_{j,i}$ need not be equal.

2.6. Co-activation networks.

Co-activation networks were defined as clusters of electrodes in matrix C with a high spike co-activation rate (Fig. 1C-D). The Louvain greedy optimization algorithm was used to partition C into electrode clusters that maximized the within-cluster co-activation rate and minimized the between-cluster co-activation rate. Clustering was performed using the Brain Connectivity Toolbox³⁰ according to the procedures of Khambhati et al.³¹ To confirm that electrode clustering was appropriate, the within-cluster and between-cluster co-activation rates were compared using the Wilcoxon rank-sum test (significance threshold, $\alpha < 0.001$).

2.7. Spatiotemporal activation sequences.

Spike co-activation typically exhibited millisecond-scale differences in peak latencies across electrodes. Each co-activation discharge could thus be represented as a spatiotemporal activation *sequence*, beginning with the earliest spike in the discharge (i.e., ‘leader’) and proceeding through downstream spikes in order of peak latency. For each network, activation sequences were extracted using a previously-described algorithm.^{11,12} Briefly, the first detected spike in the network was initialized as a the ‘leader’ of a candidate activation sequence, and all subsequent spikes with a latency < 50 ms from the leader (or < 15 ms from the preceding spike) were appended to the sequence. Sequences involving 5 electrodes were preserved. To focus on prominent spike patterns, networks with an activation rate of < 2 sequences/minute were ignored.

2.8. Quantification of regional propagation tendencies (‘Degree Preference’)

Activation sequences were represented using raster plots, with each sequence defined by the ordering of spike times in relation to the leader spike (Fig. 2A-B). To quantify electrode-level tendencies to appear upstream or downstream within activation sequences, the Degree Preference (DP) was defined:

$$DP = 100 * (\# \text{ Downstream} - \# \text{ Upstream}) / (\# \text{ Total spikes}), \quad (\text{Equation 1}):$$

where upstream spikes were those recorded earlier in sequences, and downstream spikes were those recorded later in sequences (Fig. 2B). Thus, an electrode capturing the ‘leader’ spike for all sequences would be assigned the maximal DP of +100, whereas an electrode capturing the terminal spike for all sequences would have the minimal DP of -100.

2.9. Consistency of Degree Preferences (DPs) over time

To examine the stability of regional propagation tendencies over time, DPs were first calculated from the complete set of activation sequences collapsed across segments ('Overall DPs'), and then separately for each 30-minute segment ('Segment DPs'). The Spearman rank correlation quantified the similarity between each Segment DP vector and the Overall DP vector (Fig. 2C-D). The median correlation across segments defined the 'DP-Stability' (Fig. 2D), and the interquartile range (IQR) quantified the variability across segments. To account for data sparsity, segments in which <75% of electrodes had a calculable Segment DP were excluded (for representative network featuring a window of data sparsity, see Fig. 2C-D).

For grouping across networks, electrodes were classified based on Overall DPs: Upstream (Overall DP $\geq +20$), Intermediate ($-20 \leq \text{Overall DP} < +20$), or Downstream (Overall DP < -20). For each Upstream electrode, we calculated the percentage of 30-minute segments in which an upstream Segment DP (i.e., $\geq +20$) was observed. This calculation was then repeated for Downstream electrodes. This analysis was performed to describe how reliably an individual segment captured the overall behavior of Upstream and Downstream electrodes.

2.10. Comparison of electrodes within and outside the seizure network (SZN).

To investigate the tendency for upstream spike regions to overlap with the neurologist-defined SZN, we compared Overall DPs for electrodes within the SZN ('In-SZN') to electrodes outside the SZN ('Out-SZN'). We hypothesized that In-SZN electrodes would have a higher Overall DP than Out-SZN electrodes, indicating a relative upstream activation preference. This comparison was performed at the individual and group levels using the Wilcoxon rank-sum test.

2.11. Reproducibility of individual activation sequences.

The DP analysis captured general tendencies for regions to appear at various positions within activation sequences. A complementary approach is to examine the sequence-by-sequence reproducibility of individual co-activation sequences (Fig. 3). For each network, the 'expected' sequence of network activation was defined based on the rank order of Overall DPs (Fig. 3A). Then, for each individual sequence i , the *Sequence Similarity* (S_i) was calculated as the fraction of steps in the 'observed' sequence that followed the order of the 'expected' sequence, ranging from -1 (order of the observed sequence was exactly opposite of expected order) to $+1$ (order of the observed sequence perfectly matched the expected order) (Fig. 3B-C).³² Performing this calculation for all sequences (collapsed across segments) yielded a distribution of S values (Fig. 3D). The network's *Reproducibility Index* (R) was then computed by taking the median of S values, and variability across sequences was quantified by the IQR of S values.

Non-parametric permutation testing was used to evaluate the statistical significance of the observed R (R_{orig}) for each network. To do so, the spikes times for each sequence were shuffled between electrodes to randomize the sequence order. R was then calculated on the permuted dataset, and this procedure was repeated 1,000 times to yield a distribution of

surrogate R values (R_{perm}) (see Supplemental Figure S2 for example). To account for multiple comparisons ($n = 16$ networks), the significance threshold for R_{orig} was $p < 0.003$.

2.12. Statistical reporting.

Unless otherwise noted, summary statistics are given as mean $\pm$ standard deviation (SD), and range is presented across networks. P-values correspond to two-tailed parametric tests or non-parametric permutation tests. All procedures were executed using Matlab 2016a (Natick, MA).

3. Results

3.1. Clinical characteristics.

Twelve children (9 males, mean age = 13.1 years, range = 7 – 18 years) with medically-refractory epilepsy were retrospectively studied. A variety of clinical histories, non-invasive findings, histopathologies, and electrode coverages (including both temporal and extra-temporal) were represented (Table 1). Patients were implanted with an average of 105.9 ± 16.2 subdural electrodes (range = 68 – 128 electrodes). Across patients, a mean of 8.1 ± 5.2 electrodes (range = 0 – 21 electrodes) were discarded due to artifact.

3.2. Interictal spike detection.

The mean interictal spike frequency observed across patients was 5.3 ± 2.4 spikes/electrode/minute (range = 1.9 – 9.0 spikes/electrode/minute). Following application of the non-parametric spike frequency threshold (Supplemental Table S1), the mean spike frequency for preserved electrodes was 9.2 ± 5.6 spikes/electrode/minute (range = 2.5 – 23.6 spikes/electrode/minute).

3.3. Co-activation network extraction.

Across patients, clustering of spike co-activation matrices (C) yielded a total of 16 co-activation networks for analysis (range = 1–2 per patient). Abstractly, networks were represented as nodes (electrodes) linked by edges (co-activation rate). Networks contained an average of 14.8 ± 5.1 nodes (range = 7 – 25). The within-cluster co-activation rate significantly exceeded the between-cluster co-activation rate for all networks (Supplemental Table S2), indicating appropriate electrode clustering.

3.4. Stability of Degree Preferences over segments (DP-Stability)

The DP-Stability analysis for two representative patients is shown in Fig. 4. For Patient #2 (Fig. 4A-C, left), two co-activation networks involving the left frontal and parietal lobes exhibited high DP-Stability over time. The median (IQR) rank correlation between Segment DPs and Overall DPs (i.e., DP-Stability) for Network 1 was 0.92 (0.85–0.94). Similarly, Network 2 exhibited a DP-Stability of 0.92 (0.81–0.95).

Patient #10 (Fig. 4A'-C') exhibited one very stable and one dynamic co-activation network. For the right frontal network (Network 1), DP-Stability was high (0.92, 0.86–0.96). In contrast, the right temporal network (Network 2) exhibited a pronounced shift in Segment DPs after ~24 hours, with complete inversion of upstream and downstream preferences

observed at several electrodes. The resultant DP-Stability was the lowest in the study: 0.77 (0.16–0.94).

Cohort-level results for the DP-Stability analysis are presented in Table 2 and Supplemental Figure S3. Across networks, the average DP-Stability was 0.88 ± 0.07 (range = 0.77–0.99). There was a significant negative correlation between DP-Stability and number of network nodes ($r = -0.51$, $p = 0.041$) (Supplemental Fig. S4). There was no significant correlation between DP-Stability and overall sequence frequency ($r = -0.28$, $p = 0.29$).

Across networks, a total of 59 Upstream electrodes (Overall DP +20) and 75 Downstream electrodes (Overall DP <−20) were identified. On average, Upstream electrodes exhibited an upstream Segment DP (i.e., Segment DP +20) in $77.6 \pm 19.8\%$ of segments (Fig. 4D). Upstream electrodes exhibited a downstream Segment DP in $2.1 \pm 3.5\%$ of segments. Similarly, Downstream electrodes exhibited downstream Segment DPs in $74.4 \pm 17.7\%$ of segments, and upstream Segment DPs in $4.4 \pm 7.8\%$ of segments. These results indicate that individual segments reliably captured the overall behavior of Upstream and Downstream electrodes.

At the node level, there was no significant correlation between the frequency of mono-electrode spikes (spikes/min) and Overall DP ($r = 0.10$, $p = 0.13$), nor were there any differences in spike frequency based on Overall DP category (Upstream: 10.1 ± 7.1 spikes/min; Downstream: 9.3 ± 4.8 spikes/min; Intermediate: 10.6 ± 6.1 spikes/min; one-way ANOVA, $F(2, 234) = 0.89$, $p = 0.41$).

3.5. Comparison between propagation tendencies and SZN localization.

The relationship between propagation tendencies and the neurologist-defined SZN was examined (Supplemental Table S3). In total, 86 electrodes were identified within the SZN ('In-SZN'), whereas 151 electrodes were located outside the SZN ('Out-SZN'). At the group level, there was a significant difference in Overall DPs for In-SZN versus Out-SZN electrodes, with a preference for In-SZN nodes to appear more upstream (Overall DP: $+6.4 \pm 36.1$) compared to Out-SZN nodes (Overall DP: -6.3 ± 35.8 ; Wilcoxon rank sum, $p = 0.015$). At the network-level, differences in Overall DP between In-SZN and Out-SZN nodes were not significant. However, this analysis could only be performed in 9/16 networks (56.3%) due to sparse representation of Out-SZN or In-SZN electrodes in individual networks (see Supplemental Table S3).

3.6. Sequence-by-sequence reproducibility of activation patterns

The mean sequence frequency across networks was 8.9 ± 7.4 sequences/min (range = 2.2 – 29.3 sequences/min). The *Reproducibility Index* (R) quantified the concordance between the observed and expected activation sequences, collapsing sequences across all segments. The average R_{orig} across networks was 0.45 ± 0.17 (Table 2). Non-parametric permutation testing revealed that R_{orig} was statistically significant ($p < 0.003$) for 16/16 networks. There was a significant negative correlation between R_{orig} and number of network nodes ($r = -0.75$, $p < 0.001$) (Supplemental Fig. S4). The correlation between R_{orig} and DP-Stability across networks was high ($r = 0.68$, $p = 0.004$), reflecting the fact that these metrics are closely related (Supplemental Fig. S4).

4. Discussion

Regions frequently co-activate during interictal spike discharges with millisecond-scale latencies, suggesting that spikes can propagate across the cortex. Although the distinction between primary and propagated spikes has been recognized for decades,³ relatively little is known about the mechanisms and spatiotemporal characteristics of spike propagation. In this study, we mapped the sequential activation of cortical networks during spike propagation as recorded by subdural grid and strip electrodes. Our main findings indicate that spike propagation is a spatiotemporally reproducible network phenomenon over hours to days of interictal recording.

4.1. Spike propagation as a biomarker of pathological network activity.

The concept of network-level dysfunction has been widely influential in modern epilepsy research.^{33,34} Whereas earlier disease models focused on discrete “zones” of suspected epileptogenicity,³⁵ newer models incorporate the time-varying interactions between brain regions as a fundamental driver of seizure activity.^{36,37} Propagation of epileptiform discharges has been raised as an intuitive measure of functional connectivity, offering a unique perspective of both the strength and directionality of inter-regional relationships.^{17,38} Previous studies suggest that spike propagation is a meaningful interictal biomarker of epileptic network activity, with notable work by Alarcon et al.¹⁸ showing that excision of regions appearing upstream in activation sequences correlated with higher rates of post-surgical seizure freedom. This study and others have identified a potential role of spike propagation analysis in the surgical work-up, but the clinical value of this approach is limited by uncertainties about the reproducibility of propagation patterns over time.

4.2. Spike propagation is a highly reproducible phenomenon.

The main purpose of this study was to characterize the long-term reproducibility of spike propagation sequences. In routine visual inspection of brief EEG segments, we have appreciated the tendency for regions to co-activate during spike discharges and for certain electrodes to reliably peak earlier or later in these discharges. Similar qualitative observations were reported in an influential study by Badier & Chauvel,² who examined 13 patients with temporal lobe epilepsy (TLE) undergoing stereo-EEG investigation. The need to support these visual observations using computational techniques is obvious considering the abundance of interictal spikes, long recording durations, and the large number of electrodes implanted per patient. The road towards clinical implementation of spike propagation analysis clearly demands an efficient and intuitive signal processing method.

Our approach considers each propagation event in terms of a spatiotemporal activation sequence, in which networks of cortical regions are sequentially recruited into propagating spike discharges. The method was adapted from an algorithm introduced by Kreuz et al.,³² which was originally purposed for quantifying the consistency of propagation patterns in spike train data but is generalizable to any spatiotemporal process. In our analysis, we found that spike co-activation networks were characterized by highly reproducible propagation patterns, using both a measure of sequence-by-sequence reproducibility (R_{orig}) and temporal stability of regional preferences to appear at varying latencies within sequences (DP-

Stability). Our results indicate that spike propagation patterns observed in 30-minutes epochs correlate strongly with the ‘overall’ patterns (i.e., from the complete interictal dataset), suggesting that these overall patterns can be assayed from brief epochs.

Demonstrating the reproducibility of activation sequences in this manner is novel, as no studies to our knowledge have leveraged the individual variation of spike activation sequences to generate continuous measures of propagation reproducibility. Our approach should be distinguished from previous studies by Bourien and Wendling,^{5,21} who developed an automated method to identify subsets of regions frequently co-activated during spikes. Similarly, Baud et al.¹⁰ developed a machine learning technique for unsupervised analysis of spike activity, but their approach focused on extracting archetypal propagation patterns rather than variations in individual sequences. Khoo et al.³⁹ used a waveform-averaging approach to quantify regional tendencies to appear early or late in spike propagation, but in doing so averaged away the variation between sequences that constituted the basis of the R_{orig} calculation. We suspect our methods will be particularly valuable when comparing spike patterns across states, such as interictal and pre-ictal time windows. In this setting, the variability of activation sequences may offer insights into the dynamic re-organization of the epileptogenic zone, potentially revealing changes that herald an upcoming seizure.

Interestingly, 1/16 networks (Patient #10, Network 2) exhibited an impressive shift in Segment DPs over time, with several electrodes exhibiting inversion of upstream and downstream preferences. The neurobiology underlying this transition is unclear. We speculate that time-varying factors such as sleep-wake state, medication withdraw, and temporal proximity to seizures can influence propagation patterns. The shift in Segment DPs also corresponded to an apparent state-change in the overall frequency of spike sequences (see Fig. 4A’-B’). Future studies incorporating scalp-based EEG for sleep staging, as well as detailed analysis of medication regimen and seizure proximity, will help identify factors contributing to these transitions.

The negative correlation observed between both reproducibility measures (DP-Stability and R_{orig}) and number of network nodes also warrants exploration. One hypothesis is that smaller, more spatially-contiguous networks generate more reproducible spike activation sequences, which could be tested by implementing precise methods to localize electrodes in space. Relatedly, networks that span large cortical territories may participate in multiple, partially-independent spike networks, thereby generating more heterogeneous activation sequences. Refinement of the methodology may allow us to detect networks with multiple independent (but themselves highly reproducible) propagation patterns.

4.3. Propagation tendencies relate to SZN localization, but only at the group level.

Studies comparing interictal spike propagation with seizure onset have identified a tendency for upstream regions to overlap with the SOZ.^{6,7,9} However, similar to the recent study by Maharathi et al.,¹⁷ our study provides only weak support for this observation. Specifically, differences in DPs between In-SZN and Out-SZN were only detected at the group level, and even there, the within-group variability was very high. The relationship between spike and seizure networks has been controversial for decades.⁴⁰ Previous studies have shown that complete excision of regions generating spike discharges (i.e., irritative zone) is correlated

with seizure-free outcomes,^{41,42} whereas others find that complete irritative zone resection is neither necessary nor sufficient to ensure favorable outcomes.⁴³ Network-based analysis of spike propagation is relatively understudied compared to the standard clinical approach, which emphasizes the spatial localization of spike discharges rather than spike co-activation between brain regions.⁴⁴ Mapping directional relationships using inter-spike latencies enables clinicians to distinguish between upstream and downstream regions, which may differentially correlate with the seizure onset zone.⁶ However, poor concordance between the upstream spike regions and the SZN in many patients (see Supplemental Table S3) suggests that this correlation is variable at the patient-level.

4.4. Study limitations and future directions.

Several weaknesses of the current study should be considered. Although small sample sizes are typical of pediatric intracranial EEG studies, validating our findings in a larger, preferably multi-institutional sample with a more representative gender distribution is warranted. The use of subdural electrodes prevented us from examining interactions between superficial and deep brain regions, which may reveal nuances in the propagation patterns captured at the surface.¹³ The somewhat limited acquisition frequency (200 Hz) is another source of error in detecting subtle peak-time differences between brain regions. Future work extending this paradigm to SEEG recordings with higher sampling rate is underway in our lab. All invasive EEG studies share the limitation of incomplete sampling of the epileptic brain. We note that our reproducibility analyses would translate to a host of non-invasive electrophysiology modalities, including scalp EEG and MEG, as the methodology is based generically on event times and locations. Finally, due to non-uniform availability of post-resection imaging, we did not attempt to correlate spike propagation patterns with the extent of surgical resection, which has been examined elsewhere.^{12,18}

4.5. Conclusions.

We investigated the reproducibility of interictal spike propagation in a retrospective cohort of twelve children implanted with subdural electrodes. We found that the sequential activation of network regions during spike propagation was reproducible over thousands of spike discharges across hours to days of interictal recording. These findings shed new light on long-term spatiotemporal patterns that likely constrain the network mechanisms of refractory epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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5.

Key Points

1. The long-term reproducibility of interictal spike propagation was studied in a retrospective cohort of 12 children implanted with subdural electrodes.
2. The sequential activation of network regions during spike propagation was highly reproducible over thousands of spike discharges and many hours of recording.
3. These findings shed new light on spatiotemporal dynamics that may constrain the network mechanisms of refractory epilepsy.

Significance:

Interictal spike propagation is a highly reproducible output of epileptic networks. These findings shed new light on spatiotemporal dynamics that may constrain the network mechanisms of refractory epilepsy.

Figure 1. Interictal segment selection and spike co-activation.

A) For each patient, the longest window of interictal activity without an interruption 2 hours in duration was selected and divided into non-overlapping 30-minute segments. Segments overlapping with file breaks, peri-ictal intervals, and artifact were excluded. In this example (Patient #10), 83/102 possible interictal segments (41.5 hours) were preserved. B) *Left:* Intraoperative photograph and schematic of subdural electrode implant. *Right:* Representative 10-second epoch containing spike discharges that co-occur across multiple electrodes in the frontal and temporal regions. C) Co-activation matrix (C) quantifying the

tendency for regions to co-activate during spike discharges (i.e., spike peaks separated by <150 ms). Dotted boxes indicate clustering of matrix C to reveal two co-activation networks, which are represented in the electrode schematic in (D).

Figure 2. Examination of regional propagation tendencies over time.

A) *Left*: Co-activation network encompassing 14 electrodes over the left temporal/parasyylvian regions (Patient #12). Colors denote three Upstream electrodes (Overall DP +20; red), four Intermediate electrodes (gray), and seven Downstream electrodes (Overall DP < -20; blue). *Right*: Three representative spike co-activation discharges (500-ms waveforms) revealing millisecond-scale latencies between Upstream electrodes (anterior temporal lobe) and Downstream electrodes (posterior temporal/parasyylvian). Dotted lines represent the peak time of the earliest spike in the sequence. B) Rasters from 8

representative co-activation discharges marking peak times at each electrode. The Overall DP for each electrode (*right*) reflects the tendency to appear upstream or downstream within sequences. C) Heat map encoding Segment DPs within each 30-minute segment. Warmer colors reflect a preference to appear more upstream. The heat map is sorted top to bottom according to rank order of Overall DPs. The number of sequences captured per segment is shown in gray. The first 13 segments (6.5 hours) are sparse compared to the rest of the analysis window. D) The rank correlation between each Segment DP vector and the Overall DP vector is shown. The median of correlation values is the ‘DP-Stability.’

Figure 3. Sequence-by-sequence reproducibility of network activation.

A) The rank order of Overall DPs constituted the ‘expected’ sequence of network activation. B) Three hypothetical sequences with peak times marked by dotted lines. From left to right, observed sequences exhibit decreasing concordance with the ‘expected’ sequence order. C) Calculation of the Sequence Similarity (S_i) value for each observed sequence. A point is awarded for each pairwise interaction that adheres to the expected order (e.g., for Observed (1), +1 point is awarded for the Electrode 1 → Electrode 2 interaction because Electrode 1 appears more upstream, consistent with the expected order. Zero points are awarded for ties, and -1 point is awarded when the interaction violates the expected order (e.g., Electrode 8 → 9). For each sequence (i), S_i is the sum of points divided by the maximum possible score, which is given by: $N*(N-1)/2$, where N is the number of sequence spikes. D) Histogram of S_i values for all sequences (collapsed across segments). The *Reproducibility Index* (R_{orig}) is the median S_i value.

Figure 4. Temporal stability of Degree Preferences.

Upper Left Panel: A) Two co-activation networks for Patient #2 involving the left frontal and parietal regions. B) Heat map encoding Segment DPs within each segment. Electrodes exhibit stable tendencies to appear at upstream (red), intermediate (green), or downstream (blue) positions in sequences. C) Each dot represents the correlation between the Segment DP vector and the Overall DP vector for each segment. The solid black line represents the median correlation value across segments ('DP-Stability'). In both networks, propagation tendencies were highly stable over time (DP-Stability, IQR): Network #1: 0.92 (0.85–0.94),

Network #2: 0.92 (0.81–0.95). *Upper Right Panel:* The same figure components are presented for Patient #10. Network #2 exhibited the lowest DP-Stability in the study: 0.77 (0.16–0.94). D) Upstream (n = 59) and Downstream (n = 75) electrodes were defined based on Overall DPs. On average, Upstream electrodes exhibited an upstream Segment DP in $77.6 \pm 19.8\%$ of segments and almost never exhibited a downstream Segment DP ($2.1 \pm 3.5\%$ of segments). The same pattern was seen for Downstream electrodes. Two circled Downstream electrodes are from Patient #10, Network 2 (see asterisks in B' showing these two electrodes, with inversion of upstream and downstream tendencies).

Table 1.

Clinical characteristics.

Pt	Gender / Age*	Dataset Segments / Hrs	Recording Electrodes	Rejected Electrodes	Grid Coverage	Histopathology	Engel Outcome†
01	F / 16	49 / 24.5	92	0	R FT	Tuberous sclerosis	II
02	M / 18	35 / 17.5	100	8	L FTP	FCD	IV
03	M / 11	113 / 56.5	128	5	L F	Neurofibromatosis	III
04	F / 15	59 / 29.5	68	5	R FP	FCD	III
05	M / 12	80 / 40.0	123	12	BL O	No resection	N/A
06	M / 17	77 / 38.5	100	6	L TP	Cerebral infarction	I
07	F / 11	134 / 67.0	116	7	R FTP	Cerebral infarction	I
08	M / 12	59 / 29.5	110	21	L Hemi	FCD Type IIA	I
09	M / 14	333 / 166.5	96	9	R F	FCD Type IIA	I
10	M / 7	83 / 41.5	111	10	R FTP	FCD Type IB	III
11	M / 16	70 / 35.0	118	4	R FTP	FCD Type IIA	I
12	M / 8	42 / 21.0	109	10	L FTP	FCD Type IIA	I

* Years;

† Minimum 24-month follow-up; L: Left, R: Right, F: Frontal, P: Parietal, T: Temporal, O: Occipital, BL: Bilateral; Hemi: Hemisphere; FCD: Focal cortical dysplasia

Table 2.

Reproducibility of spike co-activation patterns.

Patient	Network	Electrodes (N)	DP-Stability Median (IQR [*])	$R_{orig}^{\dagger}$ Median (IQR ^{**})
01	1	9	0.98 (0.97–1.00)	0.80 (0.73–0.90)
02	1	17	0.92 (0.85–0.94)	0.38 (0.10–0.60)
	2	13	0.92 (0.81–0.95)	0.50 (0.33–0.64)
03	1	10	0.99 (0.95–0.99)	0.76 (0.60–0.87)
04	1	11	0.86 (0.77–0.92)	0.29 (0.00–0.50)
05	1	22	0.81 (0.72–0.87)	0.20 (–0.05–0.43)
	2	25	0.84 (0.66–0.91)	0.23 (–0.06–0.44)
06	1	17	0.81 (0.64–0.91)	0.39 (0.04–0.61)
07	1	21	0.82 (0.70–0.88)	0.29 (0.00–0.50)
08	1	7	0.96 (0.89–0.96)	0.57 (0.30–0.73)
09	1	15	0.83 (0.53–0.94)	0.50 (0.13–0.70)
10	1	19	0.92 (0.86–0.96)	0.47 (0.20–0.67)
	2	15	0.77 (0.16–0.94)	0.40 (0.07–0.62)
11	1	11	0.79 (0.58–0.86)	0.52 (0.18–0.76)
	2	11	0.92 (0.75–0.97)	0.50 (0.24–0.67)
12	1	14	0.85 (0.65–0.91)	0.38 (0.00–0.62)
Group ($\mu \pm SD$)	16	237	0.88 $\pm$ 0.07	0.45 $\pm$ 0.17

^{*} IQR quantifies variability of correlation between Segment DPs and Overall DPs over segments

^{**} IQR quantifies variability of *Sequence Similarity* (S_j) values across sequences

[†] R_{orig} was deemed significant ($p < 0.003$) for 16/16 networks via permutation testing