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Delta oscillation coupled propagating fast ripples precede epileptiform discharges in patients with focal epilepsy

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\textbf{A B S T R A C T}

Epileptiform spikes are used to localize epileptogenic brain tissue. The mechanisms that spontaneously trigger epileptiform discharges are not yet elucidated. Pathological fast ripple (FR, 200–600 Hz) are biomarkers of epileptogenic brain, and we postulated that FR network interactions are involved in generating epileptiform spikes. Using macroelectrode stereo intracranial EEG (iEEG) recordings from a cohort of 46 patients we found that, in the seizure onset zone (SOZ), propagating FR were more often followed by an epileptiform spike, as compared with non-propagating FR (p < 0.05). Propagating FR had a distinct frequency and larger power (p < 1e-10) and were more strongly phase coupled to the peak of iEEG delta oscillation, which likely correspond with the DOWN states during non-REM sleep (p < 1e-8), than non-propagating FR. While FR propagation was rare, all FR occurred with the highest probability within +/− 400 msec of epileptiform spikes with superimposed high-frequency oscillations (p < 0.05). Thus, a sub-population of epileptiform spikes in the SOZ, are preceded by propagating FR that are coordinated by the DOWN state during non-REM sleep.

1. Introduction

Spontaneous seizures are the hallmark of epilepsy. Many patients with epilepsy have interictal spikes that often can be found in brain area (s) where seizures first appear, commonly termed the seizure onset zone (SOZ). Studies suggest interictal spikes could play a role in the development of seizures and their occurrence increases the likelihood for recurrent seizures(Staley et al., 2011). By contrast, other work found interictal spikes, especially in hippocampus, can reduce the likelihood for seizures in the entorhinal cortex(Avoli, 2001). These fundamentally

\textit{Abbreviations:} HFO, high-frequency oscillation; FR, fast ripple; SOZ, seizure onset zone; NSOZ, non-seizure onset zone; NREM, non-rapid eye movement sleep; SEEG, stereo EEG; GLMM, generalized linear mixed-effects model; PSD, power spectral density.

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different roles suggest that there are diverse types of interictal spikes that are associated with different brain networks and mechanisms generating them (Cohen et al., 2002; Huberfeld et al., 2011; Keller et al., 2010). Thus, identifying the different mechanisms that spontaneously generate interictal spikes will help us to understand the roles interictal spikes play in the epileptic network.

Pathological high-frequency oscillations (HFO) are also found in the epileptic brain where seizures begin as well as those associated with epileptiform discharges (Liu et al., 2022) and are believed to correspond, at the resolution of the local field potential, with abnormal bursting of predominately excitatory cells (Bragin et al., 2007, 2000). Intercital pathological HFO, which include fast ripples (FR; 200–600 Hz, 8–50 msec in duration) and some ripples (80–200 Hz 25–200 msec), are associated with the SOZ and like interictal spikes, there is some evidence pathological HFO can propagate within the SOZ (Jahromi et al., 2021; Otávalo et al., 2019). Pathological HFOs can occur alone, but often occur with interictal spikes (Jacobs et al., 2006; Urestarazu et al., 2007), especially in the SOZ (Weiss et al., 2016). In some cases, pathological HFO precede interictal spikes (Alvarado-Rojas et al., 2013). These data suggest sites where pathological HFO propagate and activate distant targets could trigger interictal spikes, but the propensity for this to happen might be greater if a broader EEG rhythm coordinated local activity levels. Broader rhythms are found during NREM periods, such as slow oscillation (<1 Hz) and delta (2–4 Hz), which are associated with alternating neuronal activation and deactivation (Levenstein et al., 2019; Sirotá et al., 2003; Steriade et al., 1993a), commonly referred to as UP and DOWN states, respectively. We and others have found pathological HFO and interictal spikes preferentially occur during the transition from the UP to DOWN state (Frauscher et al., 2015; Song et al., 2017; Weiss et al., 2020), which could be the appropriate conditions for pathological HFO propagation and consequent generation of interictal spikes.

Prior studies show during NREM sleep, the peak of depth-recorded delta wave correlates with little or no unit firing and trough of scalp-recorded delta wave (Cercea et al., 2010; Nir et al., 2011; Quyen et al., 2008). This activity likely corresponds to the DOWN (or OFF) period, though it is an estimate and doesn’t definitively identify the onset and offset of DOWN state as can be done with intracranial recording (Steriade et al., 1993b). Based on this knowledge, we measured the instantaneous phase of delta to identify DOWN (and UP) period and relate this to occurrence of pathological FR and interictal spikes (Steriade et al., 1993b). To evaluate this hypothesis, we recorded intracranial EEG from presurgical patients with medication-resistant focal seizures and analyzed the spatiotemporal properties of FR and interictal spikes during periods of NREM periods containing delta activity. We chose to focus on FR rather than ripples to reduce contamination from normal HFO (Frauscher et al., 2018), but we are aware the generation and propagation of FR could be spatially restricted compared to ripples (Bragin et al., 2002; Otávalo et al., 2019). Also, we and others use the term “propagated” to explain the time lags observed between FR (Jahromi et al., 2021; Otávalo et al., 2019), but recognize delays between FR might be due to a common synaptic driver. Thus, we provide support for our hypothesis by: 1) demonstrating evidence that FR propagate; 2) demonstrating an increased likelihood for FR propagation and strength of coupling of these events with delta at the peak phase corresponding to the DOWN state (Sirotá et al., 2003) 3) demonstrating evidence of FR propagation and association with interictal spikes.

2. Results

We identified and characterized FR during NREM sleep (Supplementary Fig. 1) in a cohort of 46 patients implanted with stereo-EEG (SEEG), which was required for pre-surgical evaluation. The focality of the patients’ seizures varied within the cohort (Supplementary Table 1) which provided some neuroanatomical diversity for our investigation of the temporal dynamics and interactions of FR generating sites to understand the mechanisms that underlie FR networks and to relate them to interictal spikes and sharp waves in the seizure onset zone (SOZ). In this study we did not further relate these networks to the surgical interventions used to treat these patients or the clinical outcomes (Supplementary Table 1).

2.1. Identifying edges with statistically significant fast ripple propagation

FR propagation has been characterized in a prior study (Otávalo et al., 2019) and we applied their method to our cohort’s iEEG data to identify, in each subject, candidate pairs of electrode contacts (i.e., edges) exhibiting the phenomenon. For each patient we computed the difference in FR onset times between each pair of electrode contacts (i.e., nodes) to identify which electrodes exhibited unidirectional FR propagation (i.e., edges; sign test, p < 0.005, FDR corrected, see methods (Otávalo et al., 2019)). We excluded FR that coincided with epileptiform spikes (Guth et al., 2021) by using the topographical analysis of the wavelet convolution and comparing the onset of the outermost closed loop contour of the FR event with the onset of the outermost open loop contour of the epileptiform discharge (Waldman et al., 2018). This was required since the propagation of the epileptiform spike could not be easily dissected from the propagation of the overriding FR.

Overall, FR propagation was rare. Among edges with FR mutual information (MI) greater than zero, in the SOZ (i.e., SOZ:SOZ), 1.32% of edges (n = 29) exhibited statistically significant FR propagation (Fig. S2A). In the NSOZ (i.e., NSOZ:NSOZ), 0.43% (n = 25) of edges exhibited significant propagation, and between SOZ and NSOZ 0.13% of the edges (n = 11) exhibited significant propagation. To examine, across all edges with a FR MI > 0, whether the territory (i.e., SOZ:SOZ) or number of FR at the FR origin predicted the likelihood of FR propagation we used a generalized linear mixed-effects model (GLMM) both predictors and the interaction were significant. The probability of propagation was highest in SOZ:SOZ edges with a higher number of FR (GLMM p < 0.005, Supplementary Fig. 2, Supplementary Table 2).

Qualitatively, in SOZ:SOZ edges, FR at the out-node (i.e., FR origin), and less often at the in-node (i.e., FR destination), preceded epileptiform spikes and sharp waves (Fig. 1, Supplementary Fig. 4A, 8A). For NSOZ:NSOZ, NSOZ:SOZ, and SOZ:NSOZ edges with significant FR propagation the associated events in the broadband iEEG were diverse (Supplementary Fig. 5,6,7). Quantitatively, for all the SOZ:SOZ edges we found a mean FR propagation velocity of 1.54 mm/s (Otávalo et al., 2019), but velocities across edges varied more than previously reported (Otávalo et al., 2019)(Figure 2A1). FR Propagation in the NSOZ:NSOZ, SOZ: NSOZ, and NSOZ:SOZ edges was at a slower velocity than the SOZ:SOZ edges (Figure 2A2,3). The neuroanatomical location of the edges showing propagating FRs were most often in limbic and frontal regions (Fig. 2B, Supplementary Fig. 3B).

Visual inspection found differences in FR morphology in the out- and in-node (Figure 1A2, Supplementary Figure 4A2,5A2,6A2,7A2,8A2) and quantitative wavelet analysis clearly shows delay between FR which argues against volume conduction (Figure 1A3, Supplementary Figure 4A3, 4B2, 5A3, 6A3, 7A3, 8A3, 8B2). Furthermore, longer propagation distance correlates with longer delay at slow velocity and at variable velocities depending on the location of the edge (Fig. 2), which is difficult to explain with volume conduction.

2.2. Differences in fast ripple properties associated with propagation

To assess whether propagation described in the preceding paragraph was due to a common synaptic driver generating FR at the in- and out-node, we compared the spectral frequency and power of each FR. We assumed if a common driver was responsible for the near simultaneous FR between the two sites, then the FR properties at each site should be similar. We analyzed all FR, and specifically FR appearing at the in- and out-node <250 ms of each other in a node pair with significant propagation (i.e., propagating FR in a propagating edge). The GLMM found
Fig. 1. Fast ripples (FR) propagate in the seizure onset zone (SOZ). (A1) Example of FR propagation (vertical line labeled “p”) in the SOZ within the left cingulate gyrus of patient 4145 after band-pass filtering 200–600 Hz. FR propagate from the out-node (LPF3–3, blue) to the in-node (LPF3–1, red), as defined by the sign test, and between other nodes not examined here. Note that FR onset in contacts from another depth electrode precede this specific FR propagation event (green vertical line). (A2) The FR propagation event in A1 at increased temporal resolution. (A3) Normalized averaged time-frequency representation of signals triggered by the onset of propagating out-node FR recorded at the out-node (top) and in-node (bottom) during the recording duration. White vertical line and arrow show delay in the normalized signal that is incompatible with volume conduction. (B1) Corresponding unfiltered iEEG of same FR in A1, note that the FR in the out node (black vertical line) precedes epileptiform discharge onset and were not classified FR on spikes by our detector. Other FR propagation events are marked by vertical lines labeled p. (B2) The FR propagation event at increased temporal resolution. (C) Raster plot of FR onset times for the in- and out-nodes (Sign test, \( p < 1 \times 10^{-10} \)). FR propagated with a mean delay of 1.6 msec across a distance of 6.4 mm. The propagating FRs (\(|\text{delay}| < 250 \text{ msec}\)) are denoted by red ticks (top). The raster plot at the time corresponding to the example in A and B at increased temporal resolution (green box, bottom). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
several factors affected spectral frequency of the FR (Fig. 3A, Supplementary Fig. 9A & Supplementary Table 3; p < 1e-6). Specifically, in the SOZ, but not the NSOZ (Supplementary Fig. 9A), non-propagating FR at the out node was associated with lower spectral frequency (Figure 3A2, ANOVA, d.f. = 326,652, F = 544.74, p < 1e-10, eta2 = 0.058), and propagating FR at the in node with short delays was associated with higher spectral frequency (Figure 3A1). The difference in spectral frequency of propagating FR in the out- and in-node at individual edges (Figure 3A1) is further evidence that the FR are not volume conducted since in that case the FR frequency would be the same. Regarding FR power, Euclidean distance rather than propagation delay was used in the GLMM because a better fit was achieved by the model (Supplementary Table 4). In the SOZ, slightly higher power at the out node and lower power at the in node was associated with FR propagation (Figure 3B2, ANOVA, d.f. = 326,652, F = 283.68, p < 1e-10, eta2 = 0.031). Higher FR power at the out node correlated with longer propagation distance, particularly in NSOZ (Supplementary Fig. 9B). These differences in FR frequency and power are more consistent with polysynaptic propagation and less consistent with a common driver. Diverse neuronal elements at in and out nodes also could contribute to differences in FR properties, though this does not readily explain the correlation between propagation delay and FR frequency or distance and FR power. Furthermore, differences in FR frequency and power, as well as duration (Supplementary Table 5, Supplementary Fig. 9), for propagating and non-propagating FR in the in- and out-node in SOZ, NSOZ, and between SOZ and NSOZ suggest different mechanisms. Because our HFO detector analyzed individual channels within patients in either a referential or bipolar montage to reduce noise (see methods) we added montage as a fixed effect with interactions to our GLMMs (Supplementary Table 6,7) and found that the effects remained statistically significant.

2.3. Delta oscillation and fast ripple coupling and propagation

The mechanisms that drive spontaneous FR generation have not yet
been fully elucidated. In studies of the kainic acid model of mesial temporal lobe epilepsy (Sheybani et al., 2019, 2018) delta oscillations (3–5 Hz) have been shown to entrain multi-unit activity and FR during the peak of the wave (Sheybani et al., 2019, 2018). Based on these findings we examined: 1) If during NREM sleep the slow/delta complex (Sirota et al., 2003) also entrains FR in recordings from patients; 2) If the strength of this delta-FR coupling relates to the likelihood of statistically significant FR propagation; 3) If the propagating FR events (i.e., appearing at the in- and out-node <250 ms of each other) exhibit distinct properties with respect to the delta-FR coupling and maybe a distinct phenomena, we distinguished the propagating FR phasors from the non-propagating events and used a Bayesian mixed-effect regression model for circular data [bpnreg] (Cremers and Klugkist, 2018) to examine if propagation (propagation status) from the out to in node (node status) influenced the preferred phase angle of coupling of the FR to Delta. This analysis was performed in edges pooled by territory and neuroanatomical region. Overall, in the SOZ and NSOZ out-node and in-node, propagating FR, across pooled edges, showed a preferred phase angle near the peak of delta (Fig. 4D, Supplementary Table 9,10). In the out-node, the preferred phase angle of the FR-Delta coupling was during the ascending portion of the wave slightly before the peak, as compared with the in-node where the preferred phase angle was nearer to the peak. For FR recorded from the SOZ in limbic structures, propagated FR occurred earlier before the peak and precessed more of the delta wave from the out- to the in-node as compared with the non-propagating events (bpmreg, Bayes Factor (BF) 2 of 2 for interaction of propagation status and node status compared to node status for predicting FR-Delta phase angle, Figure 4D1-2, Supplementary Table 9). Because the interaction of propagation status with node status was a much more significant predictor of a FR-Delta phasor angle than node status alone it confirms that propagating FR do exhibit distinct FR-Delta coupling properties.

To test if the slow/delta complex entrains FR in patients and examine the preferred phase angle of coupling we used the Rayleigh test. In the SOZ of patients, we found FR occurred near the peak of the delta wave (Rayleigh Z, p < 0.001; Fig. 4A,B1), but in the NSOZ, FR occurred at more diverse phase angles of delta (Figure 4B2). To assess whether coupling of FR with delta is associated with FR propagation, we used a GLMM to determine if FR propagation (sign test Z value) can be predicted using the coupling strength at the out-node and at the in-node (i.e., Rayleigh Z value) and territory of the edge. Results show high coupling strength at the out- and in-nodes in the SOZ predicts FR propagation (p < 1e-8; Supplementary Table 8; Fig. 4C).

To determine if the propagating FR events exhibit distinct properties with respect to the delta-FR coupling and maybe a distinct phenomena, we distinguished the propagating FR phasors from the non-propagating phasors and used a Bayesian mixed-effect regression model for circular data [bpmreg] (Cremers and Klugkist, 2018) to examine if propagation (propagation status) from the out to in node (node status) influenced the preferred phase angle of coupling of the FR to Delta. This analysis was performed in edges pooled by territory and neuroanatomical region. Overall, in the SOZ and NSOZ out-node and in-node, propagating FR, across pooled edges, showed a preferred phase angle near the peak of delta (Fig. 4D, Supplementary Table 9,10). In the out-node, the preferred phase angle of the FR-Delta coupling was during the ascending portion of the wave slightly before the peak, as compared with the in-node where the preferred phase angle was nearer to the peak. For FR recorded from the SOZ in limbic structures, propagated FR occurred earlier before the peak and precessed more of the delta wave from the out- to the in-node as compared with the non-propagating events (bpmreg, Bayes Factor (BF) 2 of 2 for interaction of propagation status and node status compared to node status for predicting FR-Delta phase angle, Figure 4D1-2, Supplementary Table 9). Because the interaction of propagation status with node status was a much more significant predictor of a FR-Delta phasor angle than node status alone it confirms that propagating FR do exhibit distinct FR-Delta coupling properties.
Fig. 4. Fast ripple (FR) are coupled to delta oscillations and this coupling increases the probability of FR propagation between edges by a distinct mechanism. (A1) Example of FR-delta coupling in the unfiltered iEEG recorded from the SOZ. Arrows point to individual FR. (A2) Trace shown in A1 after filtering in the delta (2–4 Hz) band with instantaneous phase colour coded. (B) Among the 46 patients, illustration of the aggregated nodes with statistically significant FR-delta phase coupling (Rayleigh test, \( p < 0.001 \)) in the SOZ (A1) and NSOZ (A2). The size of the node indicates the Rayleigh Z value, a measure of the strength of coupling. The colour of the node is the mean phase angle of coupling. (C) Three-dimensional bar plot illustrating the percentage of edges with FR mutual information > 0 and significant propagation (Sign Test, \( p < 0.005 \)) as a function of the out-node and in-node FR-delta phase coupling strength. Strength was measured as the Rayleigh test \( Z \) value (i.e. propagation measure) was positively correlated with higher out- and in-node FR-delta coupling strength (Rayleigh \( Z \)) in the SOZ (GLMM, \( p < 1 \times 10^{-9} \)). (D) Polar histograms quantifying pooled FR occurrence in relation to the phase of the delta wave. Note FR were measured only from edges with statistically significant propagation (\( p < 0.005 \)), and FR independent of a delta wave were excluded. FR-delta phase angle distributions were compared in the out- and in-node in limbic regions (black), frontal lobe (light blue), parietal lobe (red), temporal lobe (dark blue), and occipital lobe (green) for non-propagating events (D1) and propagating events (D2) in the SOZ. Note the difference in the scale of the polar histograms between D1 and D2. In limbic regions, the FR-delta coupling angle was best predicted by the interaction of whether the FR was recorded from the out- or in-node, and whether the FR event was propagated. (Bayesian mixed-effect regression model for circular data, relative Bayes Factor 2 of 2). In the frontal and parietal SOZ, the FR-delta coupling angle was best predicted by out- or in-node status than by the propagation status or the interaction (relative Bayes Factor ~2 of 2). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
the FR-Delta preferred phase angle more than propagation status, or the nodal status and propagation interaction (bpnreg, BF 1.98/2 frontal, BF 2/2 parietal node status compared to propagation status, Figure 4D1-2, Supplementary Table 9). Because node status was a stronger predictor of a FR-Delta phasor angle it indicates that in the frontal lobe and parietal lobe propagating FR from the out to the in node do not exhibit distinct properties with respect to FR-Delta coupling. Montage was a significant confound in the temporal neocortex SOZ (BF 2/2) and occipital neocortex (BF 1.04/0.96) bpnreg models (Supplementary Table 9) and meaningful results could not be derived.

In the NSOZ, limbic FR-Delta angle distributions were not phase locked in the non-propagating FR (Supplementary Fig. 10, Supplementary Table 10). However, at the in-node of frontal, temporal, parietal, and occipital regions, phase locking was observed for non-propagating FR (Supplementary Fig. 10, Supplementary Table 10). In the case of propagating FR in the NSOZ, FR-Delta phase locking was observed at both the in-node and out-node (Supplementary Fig. 10, Supplementary Table 10). Despite these observations at the pooled group level, our model indicated that, at the individual event level, in the NSOZ, the interaction between node status and propagation status predicted FR-Delta phase angle distributions as well as node status and propagation status alone (bpnreg, Supplementary Table 10) demonstrating that propagating FR do not exhibit strong differences with respect to FR-delta coupling.

Most of the FR in the analysis were recorded from limbic SOZ regions (Supplementary Fig. 3B). Limited sample size and sampling bias could have influenced our observations in the other neuroanatomical locations and the NSOZ. Although propagating FR often did occur at the peak of delta there. In the limbic SOZ, FR-delta coupling does appear to participate in FR generation, and be an important factor promoting propagation, independent of whether the FR is propagated. However, FR propagation appears to be a distinct mechanism because it is associated with a larger delta wave phase precession to the peak.

2.4. Interictal spikes follow propagated fast ripples at the out node

The above results indicate, in the SOZ, especially limbic areas, the peak of delta wave is associated with an increased likelihood to generate and propagate FR. The peak of the delta wave could also increase the probability to generate interictal spikes, and since interictal spikes often contain FR, we next investigated tested quantitatively whether FR propagation is associated with interictal spikes. To quantify changes in the iEEG and epileptiform activity following propagating FR, we accounted for the fact that power spectral density (PSD) and time-frequency spectrograms of epileptiform spikes demonstrate a broad band increase in power associated with the sharp transients of an epileptic spike’s positive and negative going components (Benar et al., 2010). The broad band power increases have also been associated with increased neuronal activation (Manning et al., 2009; Miller et al., 2012; Winawer et al., 2013) and measured using the aperiodic offset of the fitted PSD (Donoghue et al., 2020). In neural data, the aperiodic activity consists of exponentially decreasing power that can be fit by a 1/frequency but additionally is fit by a scalar aperiodic offset (Donoghue et al., 2020).

To assess if FR propagation is associated with the initiation of epileptiform spikes and sharp waves, we separated the propagated from non-propagated FR and created one second iEEG trials with the FR onset time aligned at 250 ms in the trial. Visual inspection confirmed our automated detection system methodology assuring that in each trial, the FR onset occurred before the initial positive or negative phase of the spike (Goth et al., 2021) (Figure 10B2, Supplementary Figure 4A5, 8A5). From these trials we measured the FR-related modulation of the post-FR iEEG signal. The strength of the modulation relates to the consistency in the onset time and morphology of the FR after-going waveform. As part of this analysis, the occurrence of the FR in the out-node was also used as a trigger for generating one second iEEG trials in the in-node to measure cross-modulation and confirm propagation. Additionally, for each set of propagated, or non-propagated, FR-triggered iEEG trials from the in-node or out-node we calculated the average fit PSD (Donoghue et al., 2020). If the post-FR signal was a strongly modulated interictal spike, and the corresponding aperiodic offset of the fit PSD was also relatively large it would demonstrate a strong association between FR and spikes. We hypothesized that this association would be stronger for the propagating FR trials compared to the non-propagating FR trials.

We found that 8 of the 29 SOZ:SOZ edges exhibited propagating FR were associated with after-going epileptiform spikes in the out- and sometimes also in-node, but in these cases, we did not observe spikes associated with non-propagating FR (Fig. 5A,B). In another 12 of the 29 SOZ:SOZ edges, a FR associated after-going spike or sharp-wave was seen following both propagated and non-propagated FR at the out- and in-node, but the modulation of the spike was larger following the propagated fast ripples (Supplementary Fig. 11,12,13). In one patient, who had longer propagation delays (Figure 2A1) and longer duration FR (Supplementary Fig. 4B), FR propagation was associated with the onset of a sharply contoured delta wave (Supplementary Fig. 14). To quantify the relationship between propagating FR and after-going spikes or sharp waves in all the 29 SOZ:SOZ edges, we examined the aperiodic and periodic parameters of the PSD fits and compared propagating FRs with non-propagating FRs in the out- and in-node. We found that in the out-node, but not the in-node, the FR-iEEG trials associated with propagating FRs had a larger aperiodic offset and peak frequency (paired t-tests, d.f. = 28, Benjamani-Hochberg adj-p = 0.03, 0.02, Cohen’s d = 0.536, 0.772, Figure 5C1, 3, Supplementary Table 11). As a control, we also performed an identical comparison of the FR-iEEG trials one second before the aligned FR events and found no significant differences (adj-p > 0.05, Fig. 5E, Supplementary Table 11). These quantitative results support that propagating FR events in the SOZ precede epileptiform spikes and sharp-waves, more often than non-propagating FR.

Since the propagating and non-propagating FR exhibited distinct properties (Fig. 3, Supplementary Table 3–5), it was required to determine whether the FR propagation status or the FR properties were more predictive of an after-going epileptiform spike. We used a GLMM to predict the aperiodic offset of individual iEEG trials in the out-node, for events in SOZ:SOZ edges, using the accompanying FR’s propagation status, power, frequency, and duration. We found that the propagation status of the FR predicted the aperiodic offset magnitude (GLMM, p < 1e-26, Supplementary Table 12), but not FR power, frequency, duration, or the interaction of FR power with propagation status (GLMM, p > 0.05, Supplementary Table 12).

When this cross-modulation and PSD fitting approach was applied to the 35 NSOZ:SOZ, SOZ:NSOZ, and NSOZ:NSOZ edges heterogeneous results were found. In one NSOZ:SOZ edge from a single patient propagating FR resulted in a more strongly modulated spike in the in-node but not the out-node (Supplementary Fig. 16), but this was not the case for other edges bordering the two territories in other patients. For the NSOZ:NSOZ edges, most commonly no epileptiform spike or sharp wave followed FR propagation (Supplementary Fig. 16A,B), although in one edge a sharp wave was seen in the out-node (Supplementary Fig. 17). Across all the NSOZ:SOZ, SOZ:NSOZ, and NSOZ:NSOZ edges, in the out- or in-node, the aperiodic and periodic parameter differences of the PSD fits between propagating and non-propagating events did not reach significance (adj-p > 0.05, Supplementary Fig. 16C, Supplementary Table 12) indicating in contrast to in the SOZ, FR propagation does not typically precede an interictal spike in the NSOZ, and in the noted examples above when it does occur, results suggest it is via a different mechanism.

2.5. Fast ripples are most probable within +/- 400 msec of HFO spikes

We used the sign test to identify FR propagation, which was rare and might not fully represent the association found between FR propagation interictal spikes if, for example, FR from several out nodes converged on
a single in node. Thus, for our last analyses we removed the constraint of the sign test and include interictal spikes containing HFO (HFO spikes) because HFO spikes are often found in the SOZ (Wang et al., 2013; Weiss et al., 2016). We found that the probability density function of either the minimum positive latency measured between FR ($n = 39,616$) and single HFO spike occurrences ($n = 84,067$, Fig 6A1), and between single FR and HFO spike occurrences (Fig 6A2) was non-uniform in almost all the patients ($\chi^2$ test, Holms-Bonferoni, $p$-adjusted $< 0.05$, Fig 6B1). Overall, FR occurred with the highest probability superimposed on the HFO spikes, but in more than half the patients, FR were otherwise most probable within 400 ms preceding an HFO spike (Fig 6B2). However, across patients, only ~4% of FR preceded spikes by <400 ms, and only ~2% of HFO spikes had FR preceding by <400 ms. We next asked if this small subpopulation of FR would most likely originate from SOZ:SOZ edges, in accord with our observations quantifying FR propagation. We found that FR preceding HFO spikes by <400 ms occurred in the SOZ more often than the other FR (Fig 6C, Supplementary Fig. 18, paired t-test, $n = 37$, $p < 1e-5$, Cohen’s $d = 0.97$). We performed boot strapping statistics to determine if the baseline rate of HFO spikes accounted for the effect and found that approximately twice as many FR occurred 400 ms prior to HFO spikes in the unshuffled data (Supplementary Fig. 19, paired t-test normalized FR occurrences latency <400 msec, $n = 46$ patients, $p < 0.05$). Curiously, we also found FR occurred most often immediately following HFO spikes as well (Supplementary Fig. 20).

3. Discussion

We provide evidence that extends previous results for rare, but significant, FR propagation during interictal NREM periods in patients with medication-resistant seizures. Although our study lacked high density microelectrode recordings that could spatially measure FR propagation at the micron level, we demonstrated evidence that, within the SOZ,
definable FR events propagate polysynaptically by demonstrating that these FR have distinct spectral frequency and power that were related to the propagation delay and distance, respectively. We found that FR propagation between the out- and in-nodes required strong FR-Delta coupling to the peak of the delta wave. Although both propagating and non-propagating events exhibited FR-Delta coupling, FR-Delta coupling was distinct for propagating FR events. Most importantly, propagating FR were more often associated with an after-going interictal spike and spectral changes consistent with increased neuronal activation. The latter results could represent one of several different mechanisms spontaneously generating interictal spikes. Overall, results suggest delta waves have differential effects in the SOZ that facilitates FR propagation and the occurrence of a sub-population of after-going inter-ictal discharges (Fig. 7).

**Fig. 6.** Irrespective of fast ripple (FR) propagation, FR probability is highest preceding epileptiform spikes with superimposed HFOs (HFO spike) and HFOs preceding these spikes most often occur in the seizure onset zone (SOZ). (A) Normalized histograms of the latency between FR events and HFO spikes in individual contacts pooled across contacts and averaged across patients (N = 46). Error bars show standard error of the mean (s.e.m). FR on spike shown in black were arbitrarily assigned a latency of zero. The histogram in A1 was calculated using latencies measured from single HFO spike events (n = 84,067), whereas A2 was calculated using single FR events (n = 39,616). Note that although the probability of FR occurrences is highest before HFO spikes, only a small proportion of HFO spikes are preceded by FR. (B) Tests of skewed HFO spike-FR latency distribution in individual patients. The proportion of patients with a non-uniform HFO spike-FR latency distribution ($\chi^2$ test, Holms-Bonferoni, p-adjusted < 0.05) was over 75% (B1), and in over 50% of patients FR were most probable within 400 ms preceding an HFO spike. For these calculations FR superimposed on spikes were excluded. (C) The mean proportion of the FR in the SOZ, compared to the non-SOZ, among FR preceding HFO spikes (<400 ms, yellow) and all other FR (blue). FR preceding HFO spikes occurred in the SOZ more often than the other FR (paired t-test, n = 37, p < 1e-5, cohen’s d = 0.97). Error bars indicate s.e.m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 7.** Graphical illustration of the mechanisms of fast ripple (FR) propagation. In the seizure onset zone, FR generating nodes receive inputs from delta oscillation drivers (green circle). The strength of coupling between delta and FR events is shown by the thickness of the green arrow. The FR occur near or at the peak of the delta oscillation corresponding to the DOWN state. The two nodes (black circles) with FR propagation across the connecting edge (black line) exhibit stronger FR-Delta coupling at the out- and in-node as compared with the node with non-propagating FR. At the out-node with FR propagation, the propagated FR is higher power and higher frequency as compared with a non-propagated FR. At the out-node a propagated FR event precedes an epileptiform discharge likely through increased neuronal excitability overcoming an inhibitory restraint mechanism. Alternatively, the epileptiform discharge may be triggered by the delta driver corresponding to the DOWN state. The propagated FR event at the out-node reaches the in-node at a relatively slow velocity through poly-synaptic conduction. At the in-node the FR is relatively lower in power and higher in frequency. FR propagation in NSOZ:SOZ, SOZ:NSOZ, and NSOZ:NSOZ edges involves diverse unresolved mechanisms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3.1. FR propagate in the SOZ and possibly outside the SOZ

Prior studies using SEEG recordings have demonstrated that both ripples (Tamiali et al., 2021; 2018) and fast ripples (Jahromi et al., 2021; Otarula et al., 2019) propagate within the SOZ. These studies did not distinguish HFOs superimposed on spikes from HFOs superimposed on oscillations and thus it is not clear if the propagation of the HFOs on spikes, in these studies, can be attributed to propagation of either the spike, the HFO, or both. In our study, we excluded FR on spike and examined only the propagation of FR on oscillations. We demonstrate that, in accord with past observations (Otarula et al., 2019), unidirectional FR propagation, at relatively slow velocities, occurs in patients with medically refractory epilepsy. A novel finding of our study was the identification of FR propagation in edges bordering or outside of the SOZ. Case by case examination of these edges demonstrated heterogeneity. Furthermore, less FR were recorded from these edges decreasing the reliability of statistically significant propagation. In SOZ:SOZ edges the FR frequency and power analysis showed that propagating FR in the out-node had higher frequency and higher power than non-propagating FR, but this was not always the case in the non-SOZ:SOZ edges. Thus, more work is required to better establish the criteria for identifying definitive propagation in non-SOZ:SOZ edges. FR outside the SOZ are important in a clinical context, since FR generating regions may represent an epileptogenic zone, necessary and sufficient for seizure generation (Nevalainen et al., 2020; Rosenow and Lüders, 2001).

3.2. Possible implications and mechanisms of FR-delta coupling

With respect to phase-amplitude coupling of HFOs and slow and delta oscillations, it is well established that ripples are bimodally coupled to slow and delta oscillations during NREM periods (Frauscher et al., 2015; Song et al., 2017; Weiss et al., 2020). In our study we refer to the slow/delta oscillation because a significant portion of the delta waves are part of the slow wave that modulates excitability during non-REM sleep (Sirotta et al., 2003). Physiological ripples occur during the transition from DOWN-UP and pathological ripples occur during the transition from UP-DOWN (Frauscher et al., 2015; Song et al., 2017; Weiss et al., 2020). Our study shows that FR, which are always pathological, occur near the peak of delta at the out-node and at the peak of delta at the in-node. This may correspond to the end of the UP-DOWN transition, and the DOWN state, respectively (Ceerész et al., 2010; Frauscher et al., 2015; Nir et al., 2011; Quyen et al., 2008; Sirotta et al., 2003). It was proposed that the UP-DOWN state is representative of increased synaptic excitability that could drive the generation of pathologic ripples and spikes (Frauscher et al., 2015). In contrast, if the FR in the propagating edges were occurring near or at the DOWN state instead than it should be associated with a paucity of neuronal activity including in intracranial human data (Nir et al., 2011). Recent evidence suggests, in the neocortex, that at the positive peak of these slow/delta oscillation inhibitory ID2/Nkx2.1 barrage firing (Chittajal et al., 2020) neuroglialiform cells (Valero et al., 2021), and select populations of pyramidal neurons (Todorova and Zugaro, 2019) are activated. If pathologically interconnected neuron (PIN) clusters that generate FR (Bragin et al., 2000) are endowed with depolarizing GABA reversal potentials (Alfonsa et al., 2015; Huberfeld et al., 2007; Pallud et al., 2014) and receive synaptic drive from these DOWN state active populations, it could potentially result in a FR. Also, this same synaptic drive to surrounding non-pathologic neurons would be hyperpolarizing and contribute to the extracellular positive peak of the slow/delta oscillation (Dubanet et al., 2021) and a surround inhibition resulting in an inhibitory restraint (Schevon et al., 2012; Trevelyan et al., 2006) (Fig. 7). In support of this notion, modeling (Demont-Guignard et al., 2012; Shamas et al., 2018) and experimental (Alfonsa et al., 2015) data support that FR may be generated by depolarizing GABAergic potential in both the local field potential and iEEG.

Additionally, propagating FR occurred earlier during the UP-DOWN transition, compared to non-propagating FR, and have greater power than non-propagating FR. This suggests that the slope of the synaptic depolarizing drive on to the PIN cluster neurons during the UP-DOWN transition is steeper for the propagating FR events. At nodes in which FR propagation did not occur, coupling with delta oscillations, corresponding to the DOWN state, was weak or absent, indicating that depolarizing inhibitory potentials of the DOWN state may not have strongly contributed to FR generation there. Also, the differences between the timing, with respect to delta peak, of the non-propagating FR in the out-node and in-node could also relate to the slope of the synaptic depolarizing drive at the two locations. In line with this hypothesis is that the FR at the in-node exhibited a smaller power.

3.3. Mechanisms of FR propagation and relation to coupling

Phase-amplitude coupling is a ubiquitous phenomenon that has been shown to mediate cognition (Canolty et al., 2006) and be important in seizure generation (Weiss et al., 2015, 2015). Herein we found that when two electrode contacts each have high FR-Delta coupling strengths, the probability of FR generated at one of these nodes propagating to the other is increased. Whether this phenomenon generalizes to other forms of phase-amplitude coupling is unknown. With respect to the synaptic mechanism of FR propagation, in the healthy hippocampus, sharp-wave ripple (SPW-R) power is correlated with the degree of SPW-R propagation across the septo-temporal axis (Patel et al., 2013). Also, higher power SPW-R propagate from dorsal CA1 to the granular retrosplenial cortex through the subiculum via the transiently increased drive of both excitatory and inhibitory neurons and their synaptic interactions (Nitzan et al., 2020; Stark et al., 2014), as opposed to via electrical transmission (Simon et al., 2014). How phase-amplitude coupling contributes to this SPW-R propagation has not yet been explored. Potentially FR propagation could occur under similar synaptic mechanisms as the SPW-R, potentially involving GABA-mediated depolarizing potentials, when a supra-threshold number of neurons are recruited. This hypothesis is supported by our observation that propagating FR in the out-node having a greater power that is proportional with the propagation distance.

3.4. FR propagation and after-going epileptiform discharges

We found in a statistically significant portion of the SOZ:SOZ edges that propagating FR in the out-node were more often followed by after-going inter-ictal discharge, relative to non-propagating FR. We quantified this observation as an increase in the aperiodic offset of the PSD fit derived from propagating as compared to non-propagating FR events at each respective edge. Thus, although FR propagation in the SOZ was relatively rare, among all the patients and their contact combinations, we still can conclude that when the FR propagation occurs the probability of a time-locked after-going epileptiform discharge increases. A failure to identify more edges with FR propagation may be due to a limitation of the sign test. The sign test assumes FR propagation is unidirectional and that each node (i.e., contact) has at most two edges (one going in, one going out). If these assumptions are not met the sign test may fail in identifying propagation between two nodes. To examine the temporal relationship between FR and epileptiform discharges a different way, the sign test was excluded, and we found that FR occur with the highest probability within +/− 400 msec of HFO spikes. However, only ~2% of all HFO spikes had a preceding FR. These results indicate that it is common for FR to precede HFO spikes, but it is rare for a HFO spike to be potentially triggered by a FR. Our findings, using HFO spikes, are similar to other data from human (Alvarado-Rojas et al., 2015), and rat brain slices treated with Mg2+ free ACSF and 4-aminopyridine examining the relationship between HFOs and all types of epileptiform discharges (Kalogeropoulos et al., 2022). At present, the mechanisms that spontaneously generate epileptiform discharges are not yet known. Single unit recording from patients with epilepsy, in vivo
and ex vivo, have demonstrated heterogeneous firing patterns of excitatory and inhibitory single units associated with discharges (Cohen et al., 2002; Huberfeld et al., 2011; Keller et al., 2010) that can sometimes be distinguished by the epileptiform discharge morphology (Cohen et al., 2002; Huberfeld et al., 2011). Thus, the FR propagation that we observed may only be associated with one type, or family, of discharge (s) and may only be found at the origin of that discharge and not sites of propagation. Notably, prior research has shown that inhibitory interneuron firing precedes certain discharges (Huberfeld et al., 2011), and this is compatible with our notion that the propagating FR preceding discharges are generated by depolarizing IPSPs. To better establish whether FR propagation is involved in generating a significant fraction of even a single type of epileptiform discharge high density LFP recordings over a large region are required to identify the origin of both the FR and the epileptiform spike consistently and accurately.

4. Limitations

A single HFO can contain a broad range of frequencies including ripple superimposed on FR (Fedele et al., 2017). We used the topographical analysis of the wavelet convolution (Waldman et al., 2018) to identify the onset times of FR to measure FR propagation. It is possible that the estimates provided by this method could prove inaccurate for certain HFO events of mixed ripple and FR frequencies, or of certain FR amplitude gradients. Another potential issue with this approach is the onset of the epileptiform spike measured as the earliest open-loop contour could occur at a latency greater than that determined as spike onset on visual review. In our GLMMs and bpnreg model, the edge number was used as the random effects term to control for the differences across nodal pairs. We did not use patient as the random effects term because FR propagation in edges varied within patients, but patients with many edges could have potentially skewed our analysis. Within all the individual recordings from patients in our dataset some contacts were recorded in referential and others in bipolar montage to improve HFO detection accuracy and reduce noise (Shimamoto et al., 2018). We included recording montage of the channel in our analysis and found that it did not influence our conclusions. Nevertheless, with respect to FR-delta coupling, montage differences could theoretically be a complicating factor. We also considered the instantaneous phase of delta in the iEEG as a correlate of the UP-DOWN transition. However, local generation of slow/delta waves (Nir et al., 2011) or pathological delta waves (Sheybani et al., 2019) could complicate this interpretation. Finally, the latencies in the <100 msec between FR and epileptiform spikes in the propagating FR SOZ SOZ edges did not match with the latencies <400 msec between the highest FR probability and epileptiform spikes in the whole dataset. This mismatch may be due to measuring the events further from the site of FR origin in the latter case but could potentially be due to two independent mechanisms.

5. Conclusion

In summary, we have found that, in rare cases, using the criteria of the sign test, FR propagate slowly (~1.54 mm/msec) within the SOZ. Differences in FR frequency and power measured at the origin and destination of the FR and measured between the propagating and non-propagating FR events suggest that FR propagate polysynaptically. FR propagation required strong FR-Delta coupling to the peak of delta (i.e., DOWN state). When FR propagation was observed the probability of a time locked after-going epileptiform discharge increased. These observations suggest that an epileptiform discharge may be triggered when the DOWN state paradoxically coordinates an increase in PIN cluster excitation that elicits a FR of a sufficient power and frequency that it can propagate polysynaptically. An after-going epileptiform discharge may be triggered by the propagating FR alone or instead the underlying DOWN state. Importantly, most discharges were not preceded by FR, and further research using higher density large-scale recordings are required to determine the overall prevalence and importance of this mechanism (Fig. 7). However, this study does suggest that networks of FR derived from FR temporal correlations (Weiss et al., 2022) could be an important substrate of the epileptic network, and that future patch-clamp, calcium and chloride imaging microscopy, microelectrode array, and optogenetic experiments in animal models of focal epilepsy could help better resolve the role of FR in spontaneously generating epileptiform discharges and potentially seizures.

6. Materials and methods

All data were acquired with approval from the local institutional review board (IRB) at Thomas Jefferson University (TJU) and University of California, Los Angeles (UCLA). Informed consent was given at each clinical center. Data were collected for research purposes without impacting diagnostic studies or clinical decision-making. Digitized data were stored in an IRB-approved database compliant with Health Insurance Portability and Accountability Act regulations.

6.1. Dataset collection

This retrospective neurophysiological study used clinical iEEG recordings from 19 patients who underwent intracranial monitoring with depth electrodes between 2014 and 2018 at UCLA and 27 patients between 2016 and 2018 at TJU for the purpose of localization of the seizure onset zone. Patients had pre-implant T1-weighted magnetic resonance imaging (MRI) for MRI-guided stereotactic electrode implantation and post-implant CT scan to localize the depth electrodes. Clinical iEEG (2000 samples per second; 0.1–600 Hz) was recorded from 8 to 16 depth electrodes each with 7 to 15 contacts using a Nihon-Koden 256-channel JE-120 long-term monitoring system (Nihon-Koden America, Foothill Ranch, CA, U.S.A.). A larger number of electrodes with more contacts were implanted at TJU than UCLA. UCLA recordings were referenced to a scalp electrode positioned at Fz in the International 10–20 System, and TJU used an electrode positioned in the white matter.

Interictal iEEG were selected from each patient during the first or second night after electrode implantation and included at least 4 h that was uninterrupted by seizures. From each of these recordings we obtained between 10 and 60 min of EEG with little or no muscle or movement artifacts that contained predominantly large amplitude, delta-frequency slow waves associated with NREM sleep. The attending neurologist determined the seizure onset zone (SOZ) from review of video-EEG of the patient’s habitual seizures. For each patient electrodes contacts labeled as the SOZ were aggregated across all seizures and did not include areas of early propagation. All remaining electrodes were labeled as non-SOZ, which in some cases, could be separated from the SOZ by sub-centimeter distances.

FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) was used on the MRI to construct individual subject brain surfaces and cortical parcellations according to the Desikan–Killiany atlas (Desikan et al., 2006). With the assistance of a neuroradiologist the Advanced Neuroimaging Tools (Avants et al., 2008) was used to coregister the CT with the MRI and the position of each electrode contact was localized to the Desikan-Killiany atlas. An in-house pipeline (https://github.com/pennmem/neurorad_pipeline) was used to transform the position of each electrode contact from individual subject space to an averaged FreeSurfer space with normalized Montreal Neurological Institute (MNI) coordinates (defined by the fsaverage brain).

6.2. HFO detection and characterization

HFOs and sharp-spikes were detected and characterized using previously published methods (Shimamoto et al., 2018; Song et al., 2017; Waldman et al., 2018; Weiss et al., 2022) implemented in Matlab (Mathworks, Natick, MA, USA)(Supplementary Methods).
6.3. Identifying fast ripple propagation and distinguishing propagated events

We computed the mutual information (MI) between FR onset for each pair of electrode contacts (Gribkova et al., 2018; Weiss et al., 2022), which could be recorded in a referential or a bipolar montage (Supplementary Methods). If MI was greater than zero, we used the sign test for zero median in Matlab (signtest.m) to assess unidirectional propagation. The MI was measured first to limit the number of potential comparisons. To perform the sign test, we subtracted the onset times of FR from one contact with the onset times of FR in the other contact using the meshgrid.m function. Differences exceeding ±250 ms were excluded (Otavulu et al., 2019). To limit false detections of propagations due to the high number of channel pairs examined, we used the false discovery rate of 0.05, and a p-value cutoff of 0.005 for the sign test was selected based on multiple comparison testing using the Benjamini-Hochberg false detection ratio (bfnr.m) for contact pairs from our dataset with over 120 FR each. This criterion was used to limit the number of comparisons defining the p-value cutoff. As some contacts had <120 FR all the edges showing significant propagation were visually inspected, and if FR propagation was not observed they were excluded. Contact pairs showing significant propagation with <120 FR each were also tested in cross-modulation experiments (see Cross-modulation). The propagated FR, in an edge showing statistically significant unidirectional propagation, were distinguished in the out-node contact (FR origin) and in-node contact (FR destination) by occurring within ±250 ms of each other.

6.4. Generalised linear mixed effects models and linear regression

To test the hypothesis that SOZ:SOZ edges with high rates of FR had the highest probability of propagating edges the territory of the edge (i.e., SOZ:SOZ) and the number of FR recorded from the out-node were fit with a generalized linear mixed-effects model (GLMM) in Matlab using the fitnlme.m function with a fixed intercept and maximum quasi-likelihood (MQL) method. The patient number for each edge was the random effects term. The fixed effects term was the Rayleigh test Z value (see (Ovarak and Fenton, 2014). Trials of unfiltered iEEG, 1 s in duration, beginning before FR onset, aligned by the onset time of the fast ripple events in the out-node at 250 ms were summed to derive a modulatory signal. The statistical significance of the modulatory signal was derived by computing 300 surrogates using phase shuffling and calculating the peak-to-peak amplitude of the randomized signals. The modulated signal was calculated by convolving each 1 s unfiltered iEEG trial, with a fast ripple occurring at 250 ms, with complex Morlet wavelets with a width of seven cycles, and a standard deviation of three cycles using Fieldtrip (http://www.fieldtriptoolbox.org/). The time-frequency representations for each trial were then averaged and normalized (using a z-score) to account for (1/f) spectral power. To measure cross-modulation and confirm that fast ripple propagation had taken place in the in-node, fast ripple events in the out-node were used to generate 1 s trials in the in-node, irrespective of whether a fast ripple had been measured at that time there, and the event-triggered methodology was applied.

6.5. Circular statistics and brain renderings

The significance and strength of FR-delta coupling was measured using the Rayleigh test (circ_rtest.m) to the FR phasors measured at a given node. The circ_mean.m function was used to measure the mean phase angles and 95% confidence intervals of the FR phasor phase angles. A Bayesian projected normal regression model for circular data (bnpreg)(Cremers and Klugkist, 2018), implemented in R, was used to fit the FR phasor angles using a random effects term of the pair number, and fixed effects predictors of the out and in status of the contact, the montage of the contact, the propagation status of the fast ripple, and the interaction of the out and in status with the propagation status. To visualize the location of nodes exhibiting statistically significant FR-delta coupling, we used brainnet viewer (Xia et al., 2013). Propagated edges in MNI space were generated in Matlab using the quiver3.m function.

6.6. Cross-modulation

To identify and characterize the low-frequency waveforms and oscillations that modulate fast ripple amplitude and are modulated by the occurrence of fast ripples, we used a event-triggered coupling methodology (Ovarak and Fenton, 2014). Trials of unfiltered iEEG, 1 s in duration, beginning before FR onset, aligned by the onset time of the fast ripple events in the out-node at 250 ms were summed to derive a modulatory signal. The statistical significance of the modulatory signal was derived by computing 300 surrogates using phase shuffling and calculating the peak-to-peak amplitude of the randomized signals. The modulated signal was calculated by convolving each 1 s unfiltered iEEG trial, with a fast ripple occurring at 250 ms, with complex Morlet wavelets with a width of seven cycles, and a standard deviation of three cycles using Fieldtrip (http://www.fieldtriptoolbox.org/). The time-frequency representations for each trial were then averaged and normalized (using a z-score) to account for (1/f) spectral power. To measure cross-modulation and confirm that fast ripple propagation had taken place in the in-node, fast ripple events in the out-node were used to generate 1 s trials in the in-node, irrespective of whether a fast ripple had been measured at that time there, and the event-triggered methodology was applied.

6.7. Power spectral density fitting and statistics

To test the hypothesis that propagating FR would be associated with an increase in the aperiodic offset of the fit power spectral density (PSD), corresponding to epileptiform spikes, as compared to non-propagating FR, a Welch PSD was applied to the 1 s fast ripple aligned trials in the out-node and corresponding 1 s trials in the in-node in Python using MNE (https://mne-tools/). Trials generated one second prior to these trials were also examined as a control. The length of the fast Fourier transform was 1000 samples, the number of overlapping points between segments was 250 samples. The periodic and aperiodic components of the PSD were fit using fitting oscillations & one over f (fooof, https://fooof-tools.github.io/foof/) (Donoghue et al., 2020) using a Lorentzian function. We fit a peak between 1 and 6 Hz. In one experiment, we derived the aperiodic and periodic parameters from the individual trials. In another experiment fooof fits were combined using the combine_fooof function to derive the average aperiodic and periodic parameters. These parameters were compared between propagating and non-propagating events using a paired t-test (ttest.m) and adjusted for multiple comparisons within each group using the Benjamini-Hochberg method (bfnr.m) with a false discovery rate of 0.05. The effect size was measured using Cohen’s D (computeCohen_d.m). A PSD was also applied to the full recording duration 20–60 min using a Welch PSD with a 20 s window.
6.8. FR and HFO spike latency measures

Within patients, and within individual contacts, the minimum positive latency value was calculated for either single FR onset times compared with all HFO spike onset times, or single HFO spike onset times compared with all FR onset times. For calculating the latency of FR preceding spikes, the timestamp of the FR onset was subtracted from the timestamp of the HFO spike onset, whereas the latency of FR following spikes was calculated by subtracting in the reverse order. The latency preceding spikes, the timestamp of the FR onset was subtracted from the timestamp of the HFO spike onset within channels but not altering the total number of HFO spikes or the FR timing. The statistical significance of the unshuffled data compared to the boosted data was assessed using a paired t-test (ttest2.m) comparing the normalized histogram for the latencies of FR following spikes, the timestamp of the FR onset was subtracted from the timestamp of the HFO spike onset across contacts and patients. A normalized histogram was derived for the latencies across individual patients. The iEEG data used for figure generation are available at https://zenodo.org/record/6451900#.YmgQie3ML9Y and. The views, opinions and/or findings contained in this material are those of the authors and should not be interpreted as representing the official views or policies of the U.S. Government or the American Epilepsy Society.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2022.105928.

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