

Supplemental Material

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Aperiodic activity reflects pathologic waveform shapes in focal epilepsy

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Aperiodic activity reflects pathologic waveform shapes in focal epilepsy

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Figures S1 to S3
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Figure S1

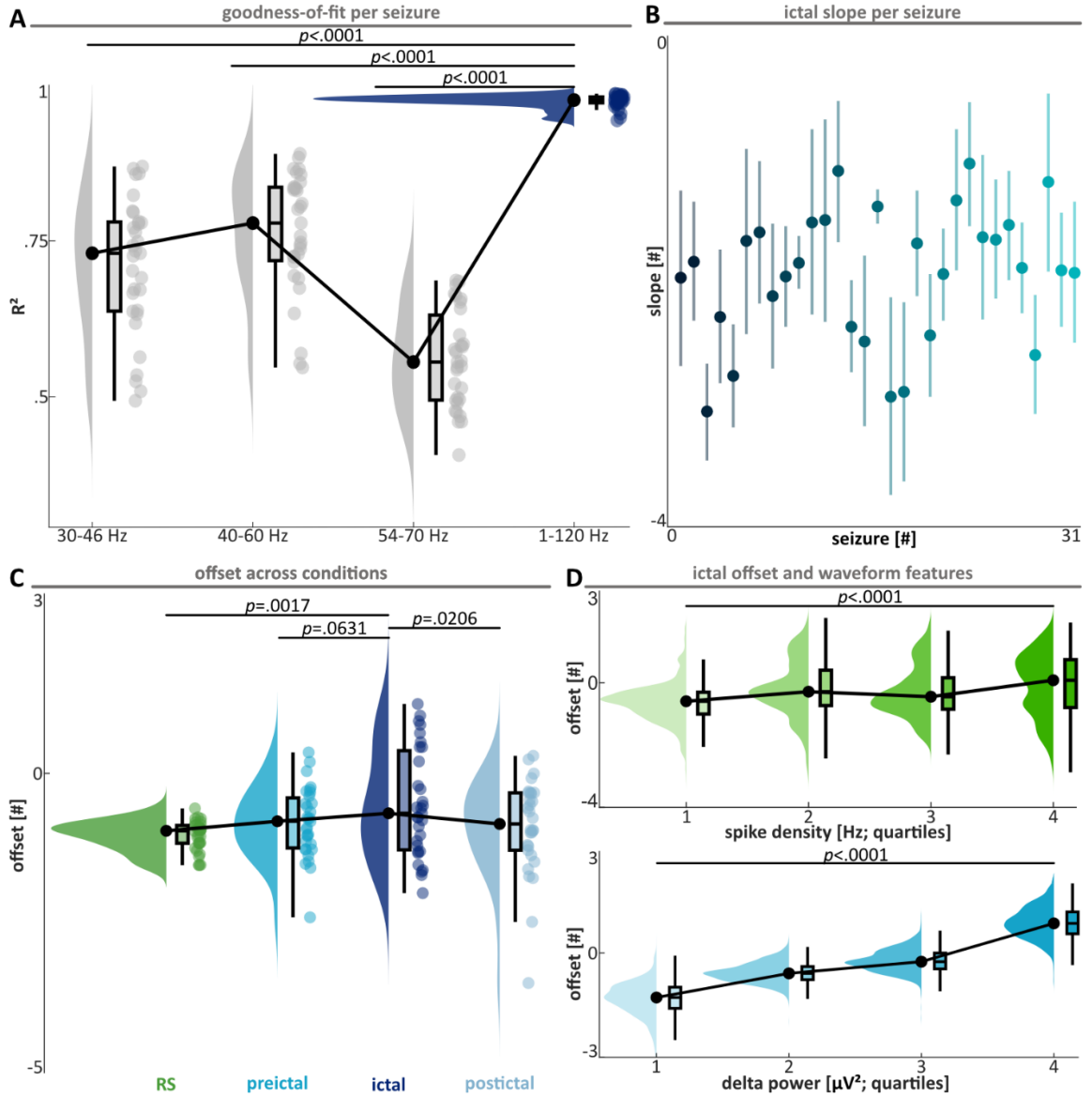


Figure S1. (A) Goodness-of-fit across frequency ranges. Mean adjusted R^2 for FOOOF model fits are significantly worse for narrow frequency windows (30-46 Hz, 40-60 Hz, and 54-70 Hz) as compared to the original broadband window (1-120 Hz; group level [$N = 31$]). P-values are reported from paired t-tests. Data are visualized as probability density functions; boxplots indicate median, 1st/3rd quartiles, and extreme values. **(B) Variability of ictal slope.** Data are visualized as mean per seizure ($N = 31$) and whiskers represent 95% confidence intervals; both computed using a permutation procedure ($N_{\text{iterations}} = 1,000$, see Methods 2.5.1). **Spectral offset dynamics during seizures. (C)** Offset per condition, showing a significant increase during the ictal condition (group level [$N = 31$]; $p = .0148$, LRT). P-values are reported from LME models. Data visualized as in panel A. **(D)** Offset as a function of spike density (*top*) and delta power (*bottom*; group level; $N_{\text{segments}} = 11,042$). P-values are reported from LRT. Data visualized as in panels A and C.

Figure S2

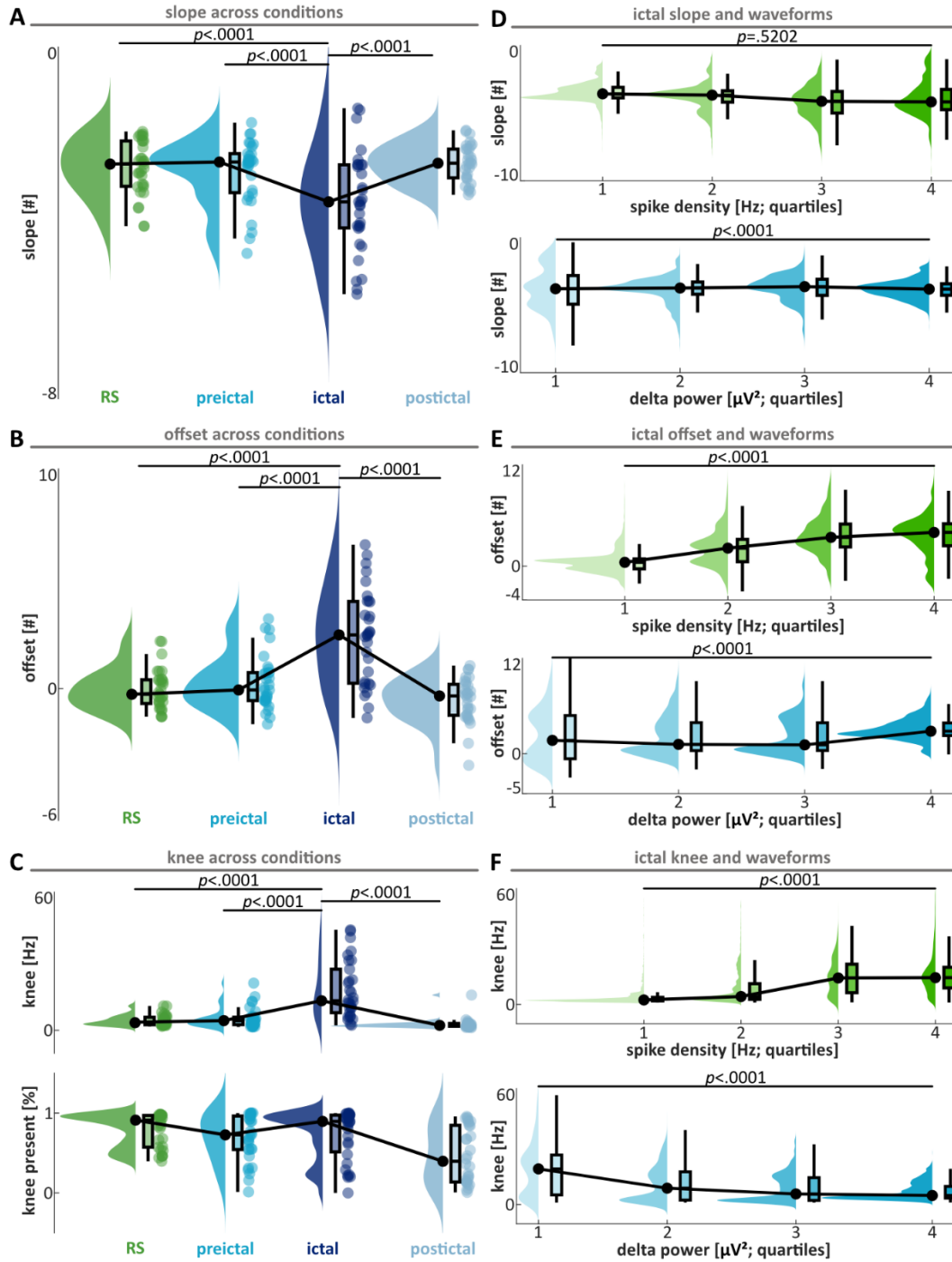


Figure S2. FOOOF with knee parameter across the entire spectrum (1-120 Hz). (A-C) Spectral slope, offset, and knee frequency per condition (group level [$N = 31$]; all $p < .0001$, LRT). P-values are reported from linear mixed effect models (LME). (C) *bottom* Percentage of segments with knee parameter per condition. Data are visualized as probability density functions; boxplots indicate median, 1st/3rd quartiles, and extreme values. (D) Spectral slope as a function of epileptic spike density (*top*) and delta power (*bottom*; group level; $N_{\text{segments}} = 11,042$). P-values are reported from likelihood ratio tests (LRT). Data are visualized as in panels A-C. (E) Same as panel E but for spectral offset. (F) Knee frequency as a function of spike density (*top*) and delta power (*bottom*; group level; $N_{\text{segments}} = 9,955$): Same convention as in panels E-F.

Figure S3

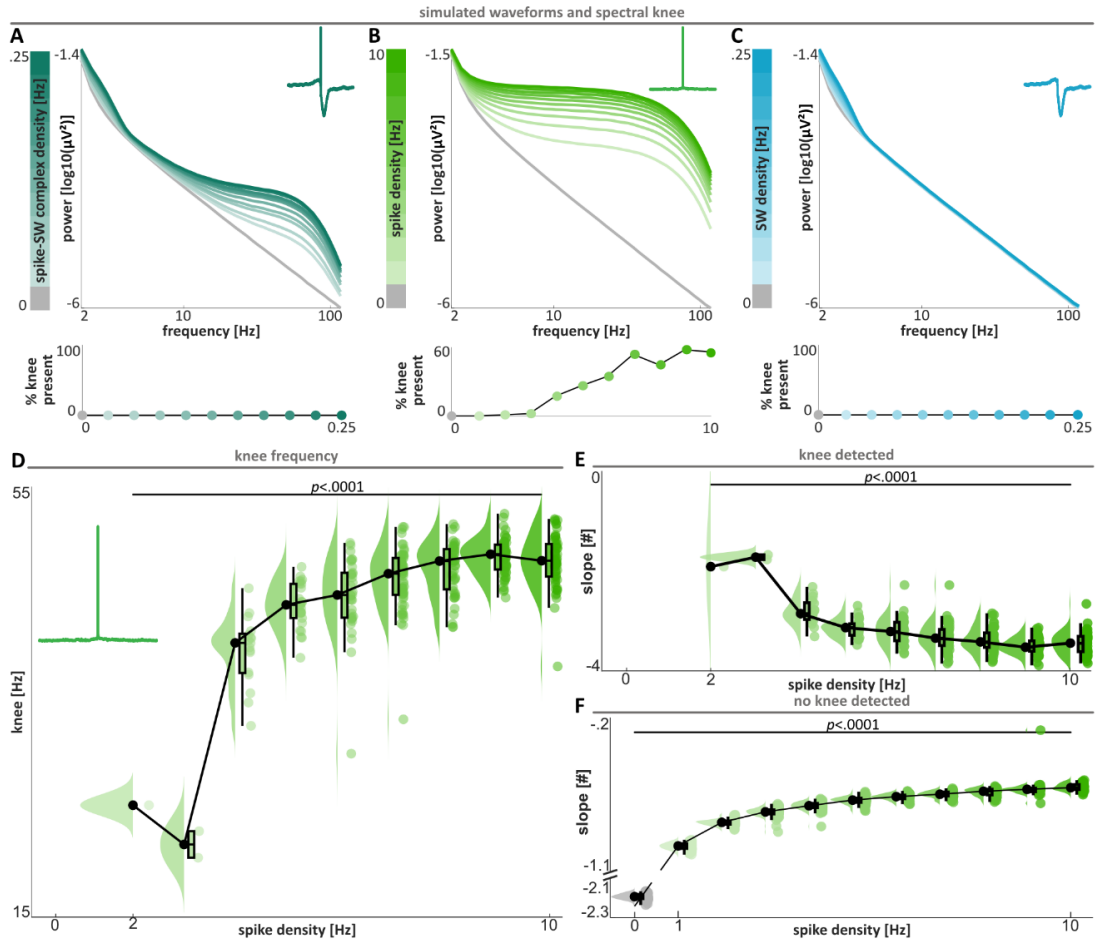


Figure S3. Knee fits in simulated pathologic activity. (A-C) Power spectrum (top) and percentage of iterations wherein a knee was detected (bottom) as a function of density of spike-slow-wave complexes (spike-SW; A), spikes (B), and slow-waves (C). Note that a knee was detected only for simulated spiking activity with densities above 2 Hz. (D) Knee frequency as a function of spike density. P-value reported from linear regression. Data are visualized as probability density functions; boxplots indicate median, 1st/3rd quartiles, and extreme values. Colors indicate spike density as shown in panel C. (E) Spectral slope as a function of spike density, for iterations wherein a knee was detected. Same conventions as in panel D. (F) Same conventions as in panel E, but for iterations wherein no knee was detected, thus yielding similar results as in Fig. 3C right.

S1: Narrow frequency ranges yield less reliable spectral parameterization

Spectral parameterization may depend on model parameters, such as the utilized frequency range (Ameen et al., 2024; Donoghue, 2024). We repeated our main analysis for various 20 Hz frequency windows (center frequencies 40, 50, and 60 Hz $\pm$ 10 Hz) utilizing a fixed linear model. Note that the 47-53 Hz range was omitted to avoid line noise (50 Hz) that may leak into adjacent frequencies through spectral smoothing (dpss, $\pm$ 2 Hz frequency smoothing). Critically, spectral parametrization is significantly more reliable when considering the full spectrum (1-120 Hz) as compared to narrower frequency ranges. This was evident through worse model fits in the 30-46 Hz range (adjusted $R^2 = .7089 \pm .0194$ [mean $\pm$ SEM], MSE = .0088 $\pm$.0003), the 40-60 Hz range ($R^2 = .7636 \pm .0173$, MSE = .0083 $\pm$.0001), and the 54-70 Hz range ($R^2 = .5592 \pm .0140$, MSE = .0083 $\pm$.0001) as compared to the full spectrum ($R^2 = .9743 \pm .0019$; MSE = .0384 $\pm$.0025; **Fig. S1A**; all $p < .0001$, paired t-test).

S2: Spectral offset tracks underlying waveform features

Upon repeating our main analysis, we find that the spectral offset differed significantly between conditions (**Fig. S1C**; $p = .0148$, LRT), being statistically significantly higher during the ictal as compared to the resting state condition (RS; $p = .0017$, $\beta = -.52$, $CI_{95} = [-.84, -.20]$, $t_{119} = -3.20$, LME) and the postictal condition ($p = .0206$, $\beta = -.38$, $CI_{95} = [-.71, -.06]$, $t_{119} = -2.35$, LME). Similarly, the offset exhibited a increase during the ictal as compared to the preictal condition, which was not statistically significant at the conventional $p = .05$ level ($p = .0631$, $\beta = -.30$, $CI_{95} = [-.62, .02]$, $t_{119} = -1.88$, LME). Examining the link between spectral offset and seizure waveform shapes, we found that the offset increased as a function of spike density (**Fig. S1D top**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = -.01$, $CI_{95} = [-.01, -.01]$, $t_{11040} = -10.7$, LME) and as a function of delta power (**Fig. S1D bottom**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = .19$, $CI_{95} = [.18, .19]$, $t_{11040} = 56.29$, LME).

Note that slope and offset showed a strong negative correlation (Spearman's rho $\rho = -.78$, $p < .0001$, $t_{30} = -13.31$; mean ρ computed across all segments per seizure and Fisher's z transformed for group-level statistics). This is expected when the power spectrum rotates, with the slope decreasing (steepening) and the offset increasing linearly. Combined with our findings for the spectral slope, these results show that both the broadband spectral slope and offset differentiate the ictal from non-ictal conditions, expressed as a flattening of the spectral slope and an increase in the offset.

S3: Spectral parameterization across the full spectrum with a knee model

Spectral parameterization may depend on model parameters, such as the inclusion of a knee parameter (Ameen et al., 2024; Donoghue, 2024). We therefore repeated the FOOOF fit for the full spectrum (1-120 Hz) and additionally included a spectral knee parameter; thus modeling the aperiodic component as a Lorentzian function. The fixed aperiodic mode was utilized when the algorithm did not converge on a knee within the considered frequency band, suggesting that a linear fit provided the best approximation of the aperiodic component. Across seizures and conditions, a knee was fitted in 67% of the data segments (**Fig. S2C bottom**). The model provided accurate fits, with an adjusted R^2 of $.9822 \pm .0010$ (mean $\pm$ SEM) and mean squared error (MSE) of $.0257 \pm .0012$.

In short, these findings support the previously reported general association between aperiodic activity and pathologic waveform shapes, albeit including a knee parameter specifically reshapes the impact of pathologic seizure activity on the spectral slope. Two explanations can account for the discrepancy between fits with and without a knee. First, when a knee is fitted by the aperiodic model, the spectral slope is estimated for the frequency range *beyond* the knee. As spectral knees during the ictal condition were detected within the 2-44 Hz range (minimum-maximum average per seizure, for details see *conditional changes* below), the frequency ranges and slope estimates diverged from our original slope analyses on broadband data (i.e., 1-120 Hz). Second, we demonstrate how the presence of ictal waveforms affects the spectral knee (for details see *effects of ictal waveforms* below). Spiking activity leads to increased power in higher frequencies, therefore increasing the knee frequency. In contrast, slow-wave activity may increase the power in lower frequencies, consequently lowering the knee frequency. Collectively, these results confirm that spectral estimates of aperiodic activity track the presence and density of seizure waveforms. Strikingly, when a knee parameter is included in the spectral parameterization, the knee frequency reflects underlying epileptic spiking activity; an effect which previously (i.e., in the fixed model) was only observed for the spectral slope. Note that the slope and knee frequency are not fully independent but show a negative correlation (Spearman's $\rho = -.61$, $p < .0001$, $t_{30} = -10.02$; mean ρ computed across all segments per seizure and Fisher's z transformed for group-level statistics), herein reflecting the mathematical relation $f_k = k^{1/\chi}$ where f_k defines the knee frequency, k the knee parameter as computed from the Lorentzian function, and χ the absolute slope or exponent.

Conditional changes.

In support of our main finding, the **spectral slope** differed significantly between conditions (**Fig. S2A**; $p < .0001$, LRT). However, the direction of the effect changed compared to our initial findings using a fixed aperiodic fit, as the spectral slope was now found to be steeper during the ictal as compared to the resting state condition ($p < .0001$, estimate of regression coefficient $\beta = .79$, 95% confidence interval $[CI_{95}] = [.47, 1.10]$, $t_{119} = 4.93$, LME), the preictal condition ($p < .0001$, $\beta = .70$, $CI_{95} = [.38, 1.01]$, $t_{119} = 4.36$, LME), and the postictal condition ($p < .0001$, $\beta = .89$, $CI_{95} = [.57, 1.21]$, $t_{119} = 5.53$, LME). Similar to our initial findings, the **spectral offset** changed significantly between conditions (**Fig. S2B**; $p < .0001$, LRT). Note that slope and offset are directly correlated in case of a rotation of the power spectrum. The increase of the spectral offset during the ictal condition was more pronounced for the knee model as compared to the fixed aperiodic model, as this effect reached statistical significance for all conditional comparisons; the resting state condition ($p < .0001$, $\beta = -2.41$, $CI_{95} = [-3.03, -1.80]$, $t_{119} = -7.71$, LME), the preictal condition ($p < .0001$, $\beta = -2.11$, $CI_{95} = [-2.73, -1.49]$, $t_{119} = -6.74$, LME), and the postictal condition ($p < .0001$, $\beta = -2.92$, $CI_{95} = [-3.54, -2.29]$, $t_{119} = -9.23$, LME).

Critically, the frequency of the **spectral knee** differed significantly between conditions (**Fig. S2C top**; $p < .0001$, LRT). The knee frequency increased during the ictal (17.42 ± 2.26 Hz [mean $\pm$ SEM]) as compared to the resting state condition (4.26 ± 0.43 Hz; $p < .0001$, $\beta = -13.16$, $CI_{95} = [-16.45, -9.87]$, $t_{118} = -7.92$, LME), the preictal condition (5.29 ± 0.80 Hz; $p < .0001$, $\beta = -12.14$, $CI_{95} = [-15.43, -8.84]$, $t_{118} = -7.30$, LME), and the postictal condition ($2.72 \pm .46$ Hz; $p < .0001$, $\beta = -14.74$, $CI_{95} = [-18.06, -11.42]$, $t_{118} = -8.80$, LME). Note that the slope and knee frequency are

mathematically directly related by the formula $f_k = k^{1/x}$ (see also section 2.1.4), thus, these do not constitute fully independent variables.

In sum, these findings show that spectral slope, offset, as well as knee frequency delineate the ictal condition, albeit that the slope steepens rather than flattens during the ictal condition when a spectral knee is fitted to the power spectrum. While all aperiodic estimates successfully delineated the ictal condition, here we focus on the spectral slope as it had been implicated in a large-scale marker of neural E-I ratio.

Effects of ictal waveforms.

To understand why including a knee parameter reverses the direction of the conditional slope changes, we determined how spectral parametrization was affected by pathologic waveform features (i.e., pathologic spikes and slow-waves). First and foremost, including a knee parameter mostly reshaped the associations between slope and offset with spike density and delta power. Contrary to our original analysis with a fixed mode, the inclusion of a spectral knee in spectral parameterization showed that spike density no longer explained the variability in the spectral slope (**Fig. S2D top**; $p_{LRT} = .5202$, LRT; $p_{LME} = .5196$, $\beta = -.001$, $CI_{95} = [-.002, .001]$, $t_{11042} = -.64$, LME). Yet, spike density was still positively associated with spectral offset (**Fig. S2E top**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = .03$, $CI_{95} = [.03, .04]$, $t_{11040} = 17.63$, LME). Contrary to the fixed aperiodic mode, increased delta power was now linked to a flatter slope (**Fig. S2D bottom**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = .06$, $CI_{95} = [.05, .07]$, $t_{11042} = 10.07$, LME), as well as a reduced offset (**Fig. S2E bottom**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = -.10$, $CI_{95} = [-.12, -.07]$, $t_{11040} = -8.09$, LME).

Second, the knee frequency tracked underlying waveform shapes; a finding which may account for the discrepancies between the fixed and the knee aperiodic modes. That is, the knee frequency increased as a function of spike density (**Fig. S2F top**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = .34$, $CI_{95} = [.33, .36]$, $t_{9953} = 42.43$, LME), but decreased as a function of delta power (**Fig. S2F bottom**; $p_{LRT} < .0001$, LRT; $p_{LME} < .0001$, $\beta = -1.06$, $CI_{95} = [-1.15, -.96]$, $t_{9953} = -22.37$, LME). To ascertain that aperiodic modulations are the direct result of the spectral decomposition of the underlying waveforms, we simulated $1/f^x$ noise and manipulated the density of pathologic spikes and slow-waves. These simulations partly confirm our findings in the real seizure data, demonstrating that the knee frequency increases as a function of spike density (**Fig. S3D**; see also section 2.6).

Combined, this set of findings shows that the spectral slope is strongly affected by the presence of pathologic waveforms in the data. Importantly, we show that the direction of the effects depends on whether a spectral knee is fitted.

S4: Results are not sensitive to segmentation parameters

To test if the results were sensitive to the utilized segmentation parameters, we repeated the analyses for different number of segments ($N_{segments} = 30$ & 40), as well as different segment overlaps (50% and 0%). Crucially, we fully replicated our findings that the slope delineates the ictal condition and reflects pathologic waveform shapes. We therefore conclude that these findings are not sensitive to the selected segmentation parameters.

Minimum number of segments.

Here, we report the statistical results for both numbers of segments ($N_{\text{segments}} = 30 \text{ \& } 40$). The spectral slope changed significantly between conditions (both $p < .0001$, LRT). The spectral slope was flatter during the ictal condition as compared to the resting state condition (both $p \leq .0301$, $\beta \leq -.17$, $\text{CI}_{95} \leq [-.32, -.02]$, $t_{110/106} \leq -2.20$, LME), the preictal condition (both $p \leq .0026$, $\beta \leq -.24$, $\text{CI}_{95} \leq [-.39, -.08]$, $t_{106/110} \leq -3.09$, LME), and the postictal condition (both $p < .0001$, $\beta \leq -.40$, $\text{CI}_{95} \leq [-.55, -.25]$, $t_{106/110} \leq -5.18$, LME). The slope flattened as a function of spike density (both $p_{\text{LRT}} < .0001$, LRT; $p_{\text{LME}} < .0001$, $\beta = .02$, $\text{CI}_{95} = [.02, .02]$, $t_{10953/10988} \geq 56.45$, LME), and steepened as a function of delta power (both $p_{\text{LRT}} < .0001$, LRT; $p_{\text{LME}} < .0001$, $\beta = -.09$, $\text{CI}_{95} = [-.10, -.09]$, $t_{10953/10988} \leq -37.62$, LME). Note that two seizures and one patient, or three seizures and 2 patients were excluded due to insufficient length of the ictal condition for a minimum of 30 and 40 segments, respectively. In sum, these supplementary analyses demonstrate that our originally reported association between spectral estimates and pathologic waveforms was not affected by the number of segments considered for analysis.

Percentage overlap between segments.

Here, we report the statistical results for both 50% and 0% overlap between segments. The spectral slope differed significantly between conditions (both $p < .0001$, LRT). The spectral slope was flatter during the ictal condition as compared to the resting state condition (both $p \leq .0051$, $\beta \leq -.21$, $\text{CI}_{95} \leq [-.36, -.07]$, $t_{117} \leq -2.86$, LME), the preictal condition (both $p \leq .0003$, $\beta = -.29$, $\text{CI}_{95} = [-.44, -.14]$, $t_{117} \leq -3.77$, LME), and the postictal condition (both $p < .0001$, $\beta = -.46$, $\text{CI}_{95} = [-.61, -.31]$, $t_{117} \leq -6.00$, LME). During the ictal condition, the slope flattened as a function of spike density (both $p_{\text{LRT}} < .0001$, LRT; $p_{\text{LME}} < .0001$, $\beta = .02$, $\text{CI}_{95} = [.02, .02]$, $t_{2209/1100} \geq 17.67$, LME), and steepened as a function of delta power (both $p_{\text{LRT}} < .0001$, LRT; $p_{\text{LME}} < .0001$, $\beta \leq -.09$, $\text{CI}_{95} \leq [-.10, -.08]$, $t_{2209/1100} \leq -11.83$, LME).

S5: Knee frequency tracks density of *simulated* epileptic spikes

Spectral parameterization may depend on model parameters, such as the inclusion of a knee parameter (Donoghue, 2024). We therefore repeated the analyses on the simulated data including a knee parameter into the model. In short, this Supplementary analysis showed that the knee frequency tracks the spike density, thus replicating findings from the patient's seizure recordings (see **Text S3** and **Fig. S2**).

Specifically, spectral knees were detected when adding simulated spikes to $1/f^\alpha$ noise characterized by a spectral slope of -2 (**Fig. S3B,D-E**). Contrary to the fixed mode, dense spiking activity significantly steepened the slope (**Fig. S3E**; $p < .0001$, slope $-.21 \pm .01$ [mean $\pm$ SEM], $t_{287} = 48.84$, $R^2 = .69$, linear regression). Crucially, the knee frequency significantly increased with spike density (**Fig. S3D**; $p < .0001$, slope $3.14 \pm .13$ [mean $\pm$ SEM], $t_{287} = 25.01$, $R^2 = .68$, linear regression). Note that the linear regression models utilize weighted least squares, wherein weights are proportional to the inverse of the number of samples at each density level.

No spectral knees were detected when simulated spike-slow-wave complexes (spike-SW) or slow-waves were added to $1/f^\alpha$ noise (cf. power spectra in **Fig. S3A** and **Fig. S3C**). The aperiodic estimates are therefore equal to the fixed mode (see **Fig. 3B,D**). Note that inspection of power

spectra in **Fig. S3A** suggests that spike-SW complexes induce a spectral knee which was not picked up by the algorithm, thus, representing a limitation to the present analysis.