Supplementary Figures

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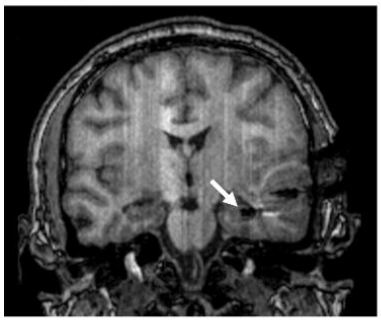

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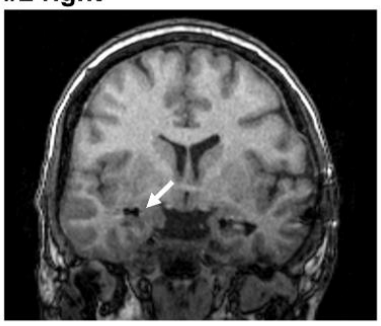

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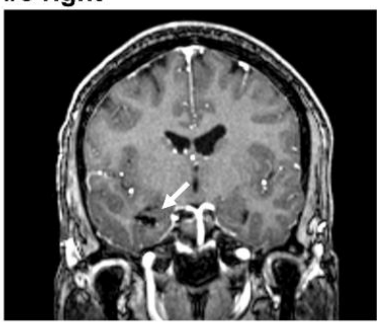

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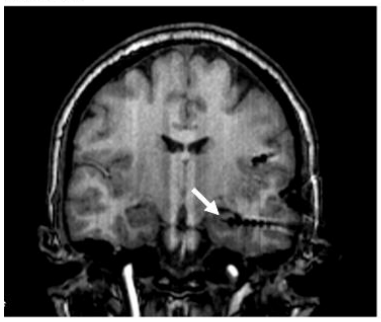

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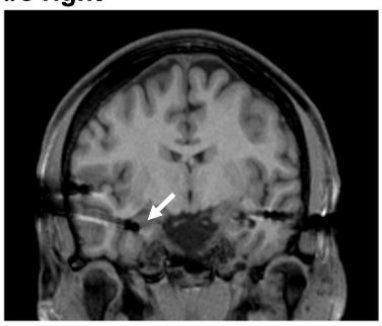

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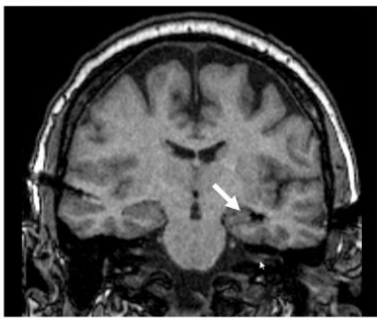

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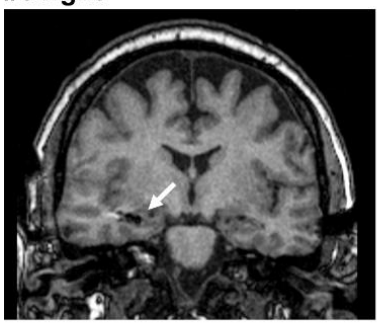

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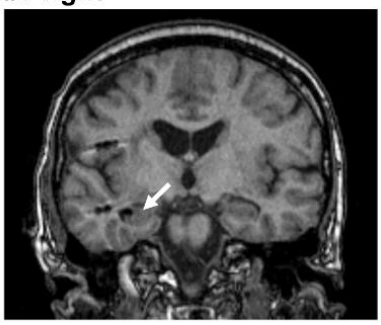

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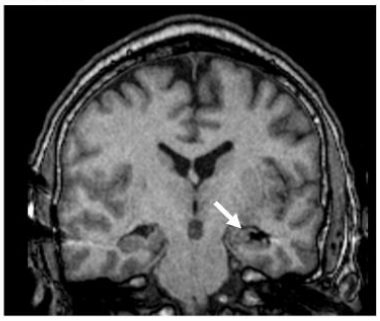

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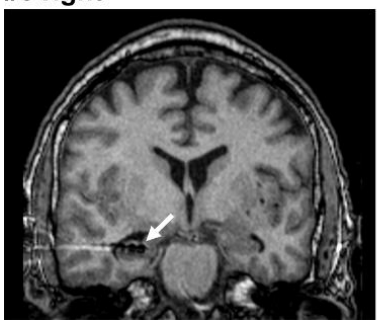

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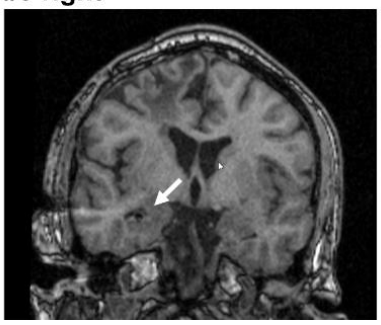
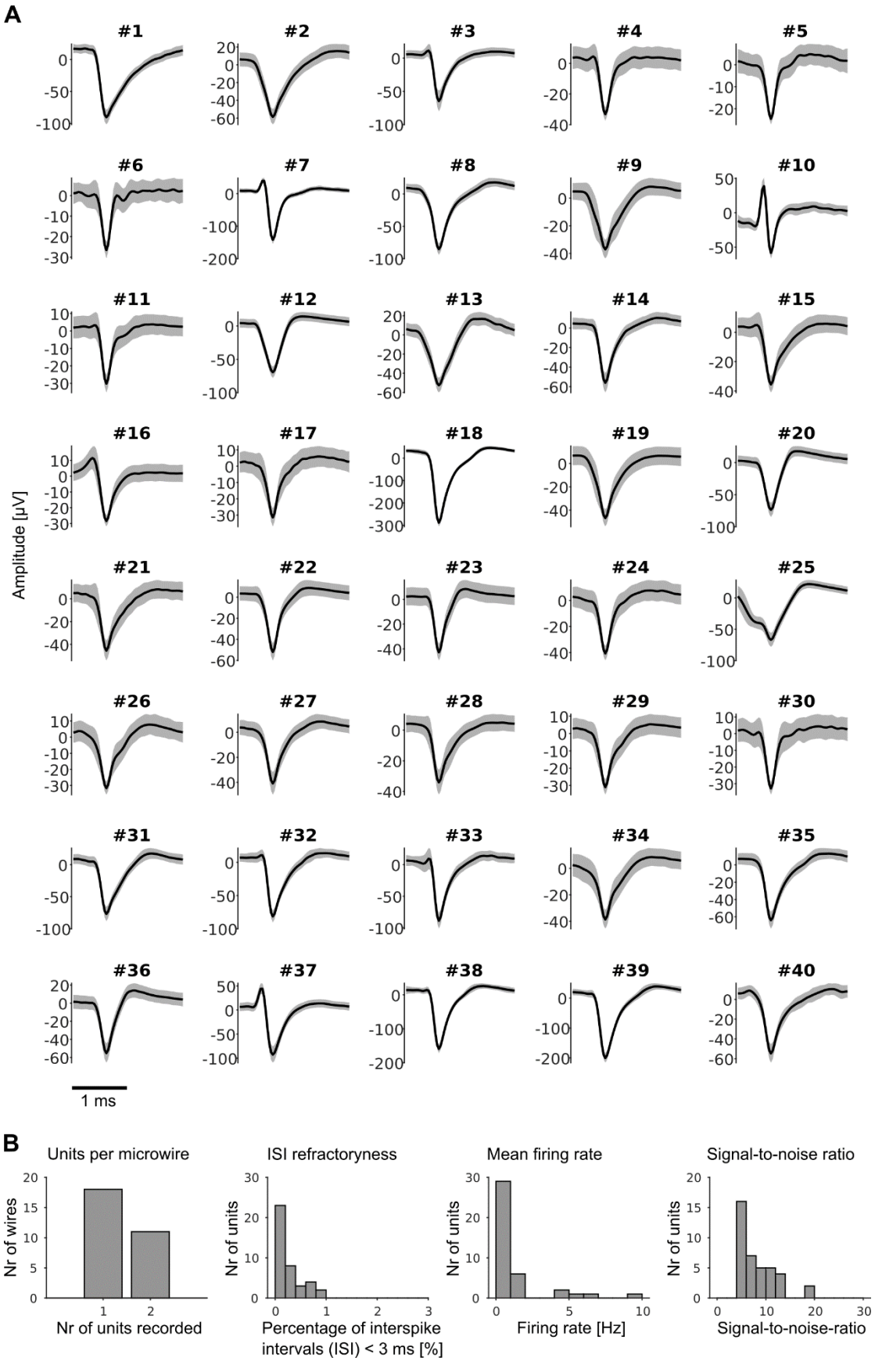


Supplementary Figure 1. Electrode localization. Coronal T1-weighted sections from post-implantation MRI, displayed in radiological convention. Patient ID refers to table 1, which contains clinical information. Arrows indicate the distal contacts of hybrid depth electrodes implanted into the anterior hippocampus.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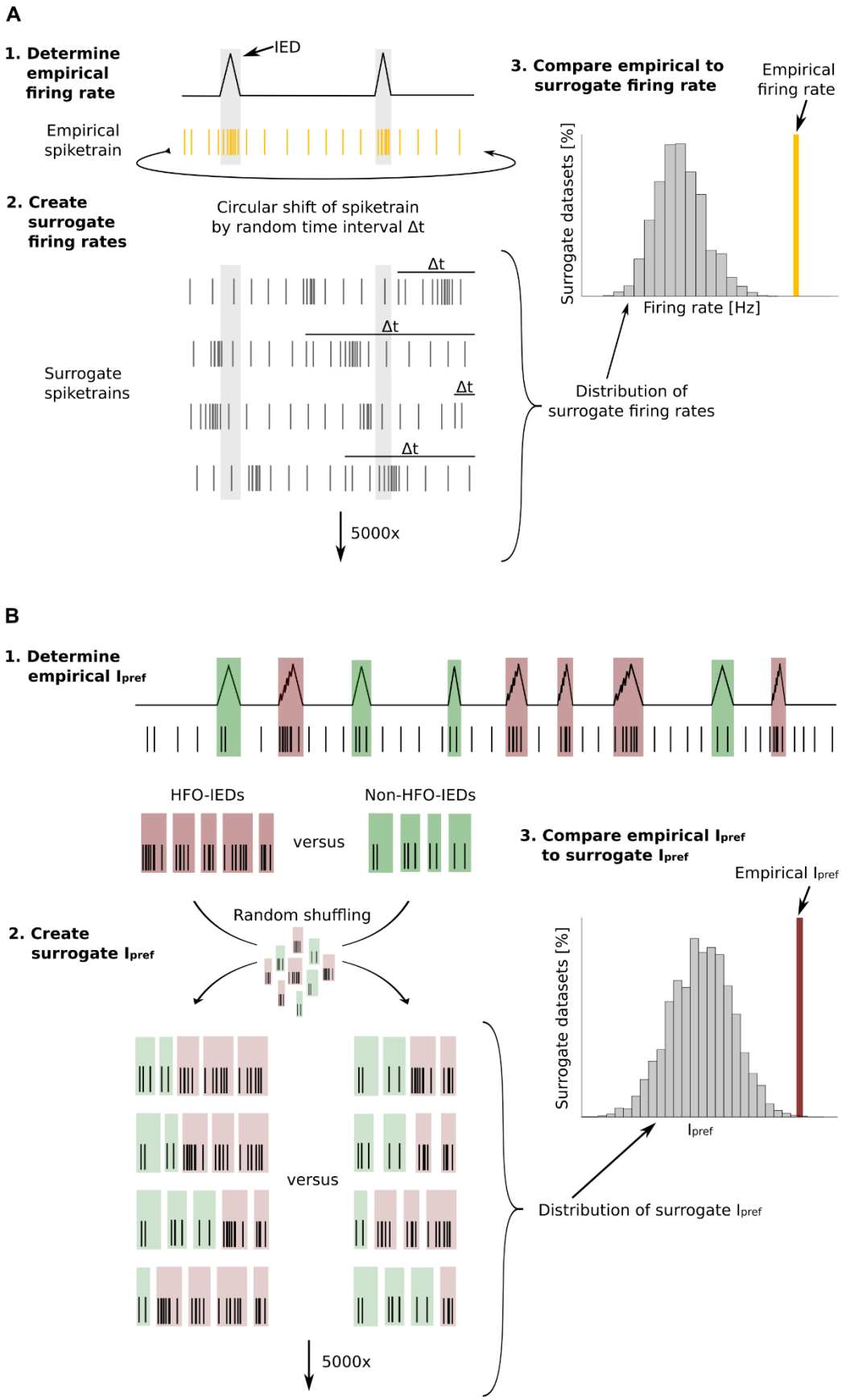
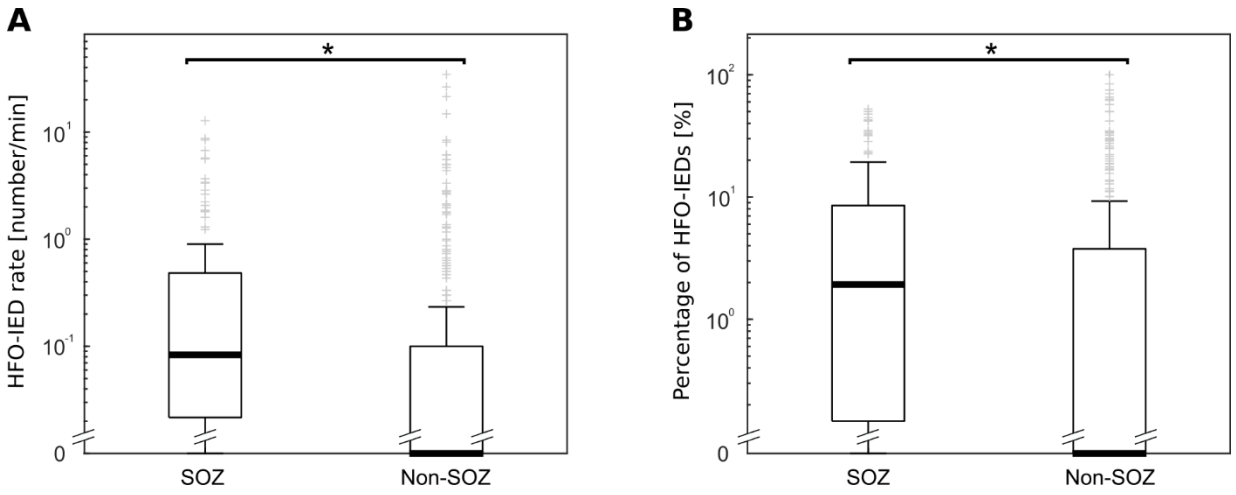
Amplitude [µV]
1 ms
B
Units per microwire
Nr of wires
Nr of units recorded
ISI refractoryness
Nr of units
Percentage of interspike intervals (ISI) < 3 ms [%]
Mean firing rate
Nr of units
Firing rate [Hz]
Signal-to-noise ratio
Nr of units
Signal-to-noise-ratio

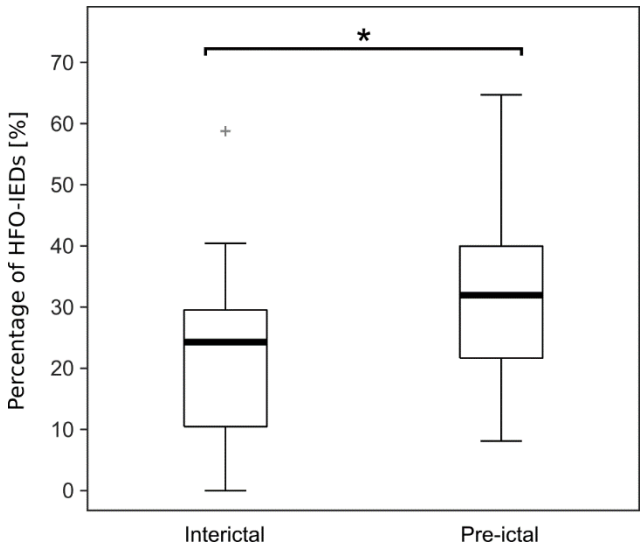
Supplementary Figure 2. Single unit quality. (**A**) Mean waveform of each single unit. Gray shading indicates standard deviation. (**B**) Additional information and quality metrics. From *left* to *right*: Histogram of how many single units were detected on each microwire (only microwires with at least one single unit are shown); histogram of the percentage of interspike intervals shorter than 3 ms. All single units have less than 1% interspike intervals < 3 ms; histogram of mean firing rates; histogram of signal-to-noise ratios, i.e. the ratio between the peak amplitude of the mean waveform and the standard deviation at the first sampling point (noise).


A
1. Determine empirical firing rate
IED
Empirical spiketrain
2. Create surrogate firing rates
Circular shift of spiketrain by random time interval Δt
Surrogate spiketrains
Δt
Δt
Δt
Δt
5000x
3. Compare empirical to surrogate firing rate
Empirical firing rate
Surrogate datasets [%]
Firing rate [Hz]
Distribution of surrogate firing rates
B
1. Determine empirical Ipref
HFO-IEDs
versus
Non-HFO-IEDs
2. Create surrogate Ipref
Random shuffling
versus
5000x
3. Compare empirical Ipref to surrogate Ipref
Empirical Ipref
Surrogate datasets [%]
Ipref
Distribution of surrogate Ipref

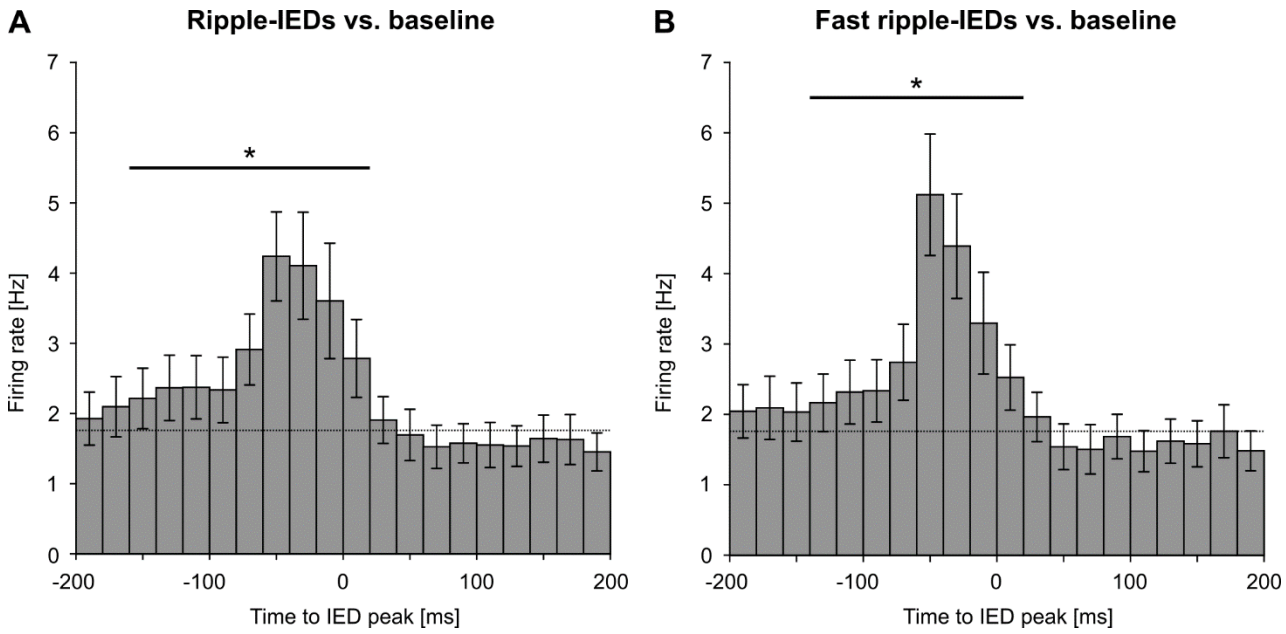
Supplementary Figure 3. Statistical hypothesis testing with surrogate data. (**A**) To test whether a unit's firing during IEDs differed significantly from baseline, we first determined its firing rate during IEDs ('empirical firing rate'; step 1 to 3). Next, we shifted this unit's empirical spike train circularly by a random time interval (delta t) to create a surrogate spiketrain, and again computed the firing rate during IEDs ('surrogate firing rate'; step 4). This step was repeated 5000 times (step 5) to compute a distribution of surrogate firing rates (step 6). Finally, the empirical firing rate was compared to the distribution of surrogate firing rates. A unit's firing rate was considered significantly altered if its empirical firing rate ranked above 97.5 % or below 2.5 % of all surrogate firing rates. (**B**) To test whether a unit's firing rate differed significantly between HFO- and non-HFO-IEDs, we first computed its preference indicator I_{pref} (see methods section for details, 'empirical I_{pref}'; steps 1 to 4). Surrogate datasets were then created by random shuffling of event labels (step 5), i.e. each detected event (or, more precisely, the corresponding interval of the unit's spiketrain) was randomly assigned to group 'HFO-IEDs' or group 'non-HFO-IEDs' without changing the number of events per group. This procedure was repeated 5000 times (step 6) to compute a distribution of surrogate I_{pref}. Finally, the empirical I_{pref} was compared to the distribution of surrogate I_{pref} (step 7). A unit's firing rate was considered significantly altered if its empirical firing rate ranked above 97.5 % or below 2.5 % of all surrogate firing rates.



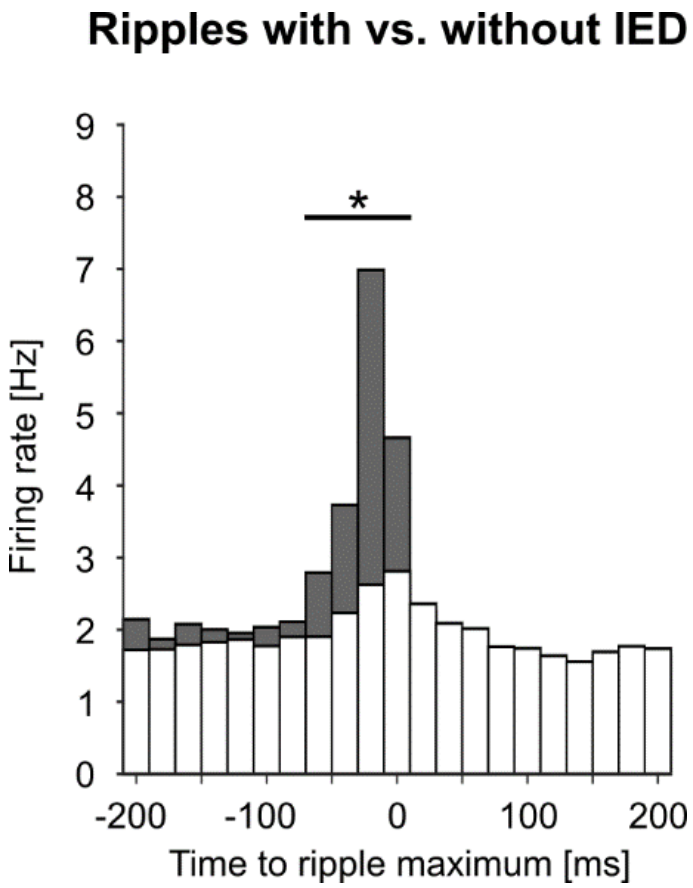
Supplementary Figure 4. HFO-IEDs are specifically associated with the seizure onset zone (SOZ). (A) HFO-IED rates were significantly higher inside the SOZ ($p < 0.001$). **(B)** The percentage of IEDs carrying an HFO was significantly higher in SOZ channels ($p < 0.001$).



Supplementary Figure 5. The percentage of HFO-IEDs is increased prior to seizures. The percentage of IEDs with an HFO was significantly higher in the 5-minute interval immediately before seizure onset as compared to a control interval 20 minutes before seizure onset ($p < 0.01$).



Supplementary Figure 6. Neuronal firing during ripple- and fast ripple-IEDs. Histograms show mean multi-unit firing rate (n = 108 multi units) and standard error of the mean (error bars) during **(A)** ripple-IEDs and **(B)** fast ripple-IEDs. Horizontal bars and asterisks indicate significant increases compared to baseline ($p < 0.001$).



Supplementary Figure 7. Neuronal firing during ripples with and without IED. Histogram showing mean multi-unit firing rates (n = 108 units) during ripples with (grey) and without (white) associated IED. Direct comparison reveals significantly higher firing in ripples with IED (horizontal bar, cluster-based permutation test, $p < 0.001$).

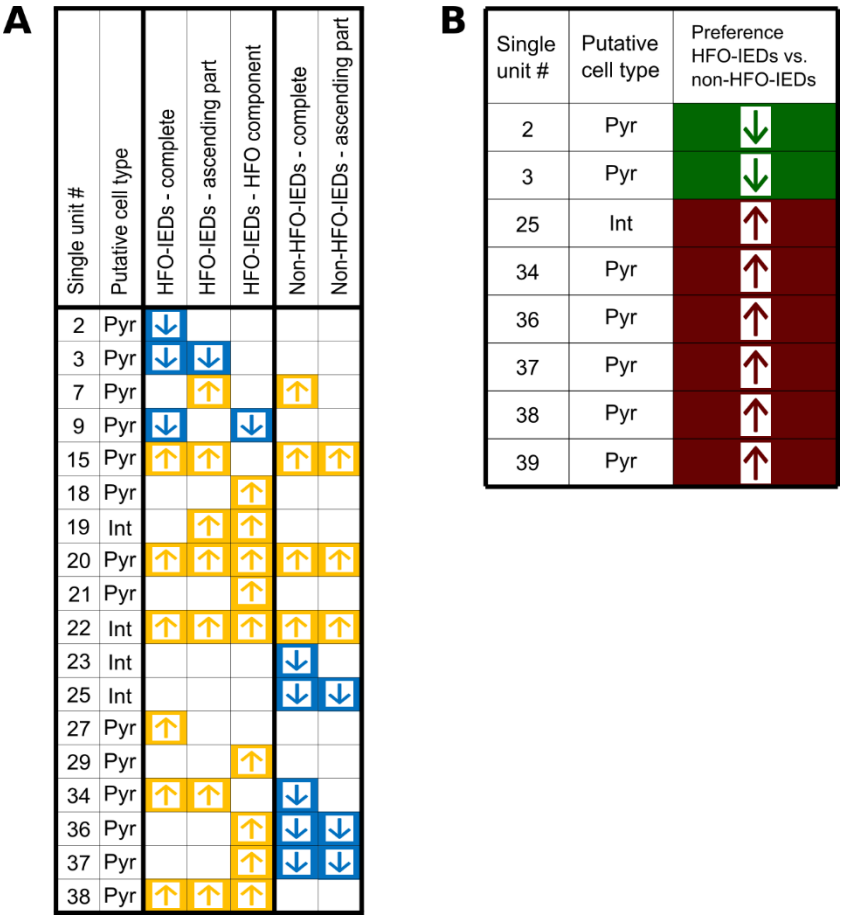
A

Patient ID / hemisphere	HFO-IEDs	Ascending part HFO-IEDs	HFO component HFO-IEDs	Non-HFO-IEDs	Ascending part Non-HFO-IEDs
8 / L					↑
8 / L	↓	↓			
6 / R*	↓				
8 / L	↓				
1 / L*	↓	↓			
4 / L*		↑		↑	
8 / L*	↓		↓		
5 / L			↑		
1 / L			↑		
3 / R	↓	↓	↓		
1 / L		↓			
9 / R*	↑	↑		↑	↑
5 / L		↑	↑		
4 / L*			↑		
6 / L*		↑	↑		
7 / R*	↑	↑	↑	↑	↑
8 / R	↑	↑	↑		
4 / L*			↑		
7 / R*	↑	↑	↑	↑	↑
8 / R*				↓	
6 / L			↑		
9 / R*				↓	↓
5 / L		↑	↑		
4 / L			↑	↓	↓
7 / R	↑	↑	↑	↑	↑
2 / R*	↑				
6 / R	↓		↑	↓	↓
8 / R	↑	↑	↑		
1 / L*			↑		
4 / L			↑	↓	
5 / L	↑	↑	↑		
5 / L	↑	↑	↑		
5 / L	↑	↑	↑	↑	↑
7 / R	↑	↑	↑	↑	↑
7 / R	↑	↑			
7 / R	↑	↑	↑	↑	↑
7 / R	↑	↑	↑	↑	↑
5 / L	↑	↑	↑		
6 / L		↑	↑		
2 / R	↑				
9 / R*	↑	↑		↓	
4 / L	↑	↑	↑	↓	
4 / L	↑	↑	↑		
1 / L	↑		↑	↓	
4 / L*			↑	↓	↓
6 / L	↑	↑	↑		
5 / L	↑	↑	↑		
5 / L		↑	↑		
7 / R	↑	↑	↑	↑	↑
1 / L			↑	↓	
7 / R	↑	↑	↑		
5 / L			↑		
4 / L	↑	↑	↑		
6 / R*			↑	↓	↓
6 / R	↑	↑	↑	↓	↓
4 / L	↑	↑	↑		
9 / R			↑		
7 / R*	↑	↑	↑		
6 / L			↑		
6 / L			↑		

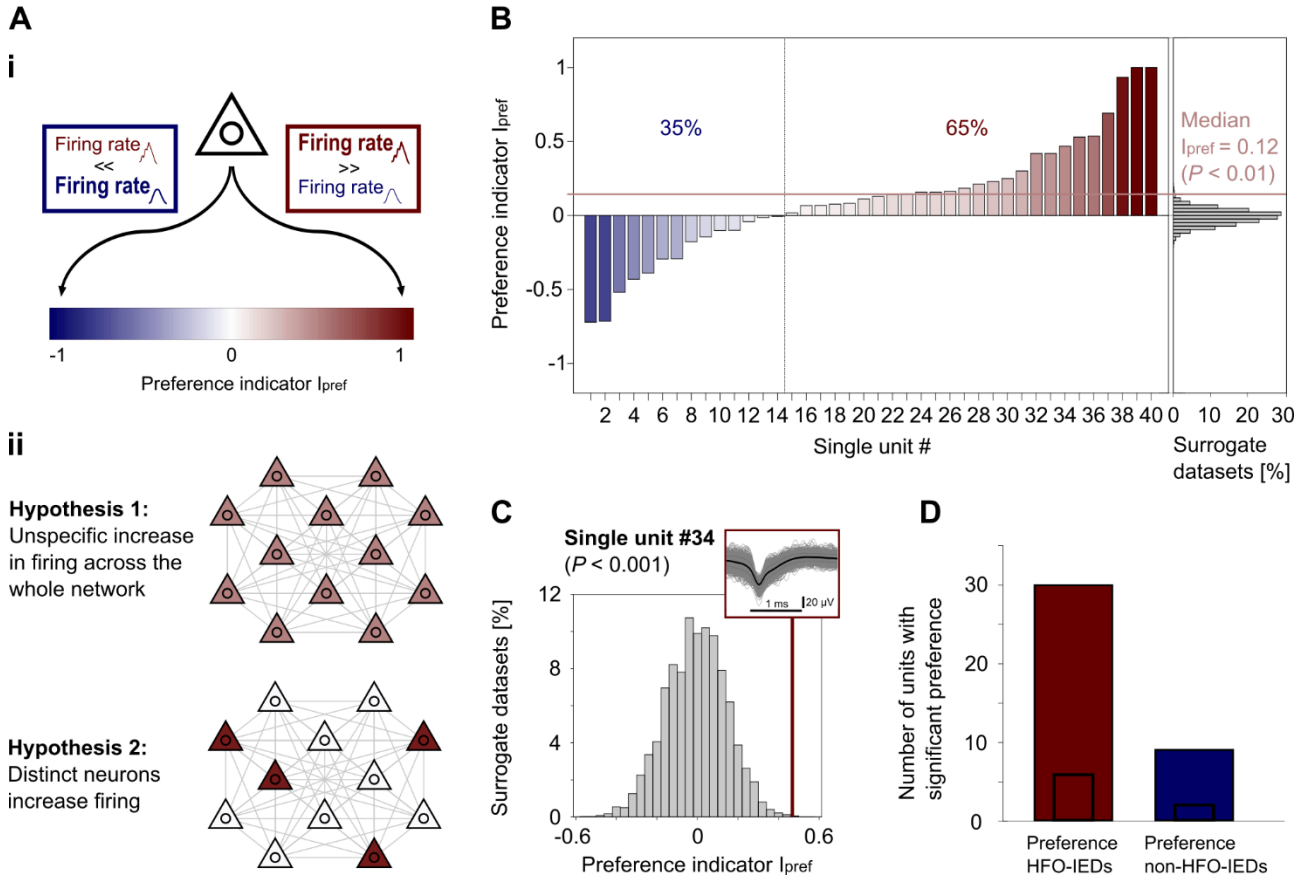
B

Patient ID / hemi-sphere	HFO-IEDs vs. Non-HFO-IEDs
8 / L	↓
8 / L	↓
8 / L	↓
6 / R*	↓
8 / L	↓
8 / L	↓
1 / L*	↓
2 / R	↓
9 / R	↓
9 / R*	↑
4 / L	↑
7 / R	↑
6 / R	↑
4 / L	↑
5 / L	↑
5 / L	↑
5 / L	↑
7 / R	↑
7 / R	↑
7 / R	↑
5 / L	↑
9 / R*	↑
4 / L	↑
1 / L	↑
4 / L*	↑
6 / L	↑
5 / L	↑
5 / L	↑
7 / R	↑
1 / L	↑
7 / R	↑
5 / L	↑
4 / L	↑
6 / R*	↑
6 / R	↑
4 / L	↑
9 / R	↑
7 / R*	↑
7 / R*	↑

Supplementary Figure 8. Multi-unit firing behavior. (A) Multi-unit behavior (n = 108 units) during different parts of HFO- and non-HFO-IEDs, comparison to baseline firing rates. Orange upward arrows indicate a significant increase in firing, blue downward arrows a significant decrease. Note that many units increased firing specifically during the HFO component (44 units ($p < 0.001$), only 2 decreased) or ascending phase (31 units ($p < 0.001$), only 4 decreased) of HFO-IEDs. The numbers of significantly increased and decreased units were different between HFO- and non-HFO-IEDs ($p < 0.05$, Fisher's exact test). (**B**) Multi units with significantly different firing between HFO- and non-HFO-IEDs. Arrows indicate higher firing during HFO-IEDs (red upward) or during non-HFO-IEDs (green downward). Note that 30 ($p < 0.001$) of 39 units preferably fired during HFO-IEDs. Patient ID refers to table 1 which contains additional patient information. Abbreviations: R, right; L, left.



Supplementary Figure 9. Single-unit firing behavior in detail. (A) Single-unit (n = 40 units) behavior during different parts of HFO- and non-HFO-IEDs, comparison to baseline firing rates. Orange upward arrows indicate a significant increase in firing, blue downward arrows a significant decrease. (**B**) Single units with significantly different firing between HFO- and non-HFO-IEDs. Arrows indicate higher firing during HFO-IEDs (red upward) or during non-HFO-IEDs (green downward).



Supplementary Figure 10. Colorblind-friendly version of Figure 4. See main text for a detailed description.

Supplementary Tables

Group	Number of units with firing rate above baseline	Number of units with firing rate below baseline
HFO-IEDs	60 (56 %)	48 (44 %)
non-HFO-IEDs	42 (39 %)	66 (61 %)

Supplementary Table 1. Neuronal firing during HFO- and non-HFO-IEDs. During HFO-IEDs, the majority of units fired above baseline, whereas during non-HFO-IEDs, the majority fired below baseline ($p < 0.05$, Fisher's exact test; n = 108 multi units).

Number of units with preference for HFO-IEDs	Number of units with preference for non-HFO-IEDs
77 (71 %)	31 (29 %)

Supplementary Table 2. Neuronal firing in HFO- vs. non-HFO-IEDs. The majority of units had higher firing rates during HFO-IEDs ($p < 0.001$, binomial test; n = 108 multi units).

Supplementary Text

Automated detection of IEDs and HFOs with the Delphos detector

For additional analyses, IEDs and HFOs (80-512 Hz) were identified in bipolar montages using the Delphos detector (Version 1.0.1)[33,34] within the open source software Anywave[63]. A detailed description of the algorithm can be found in the original publications. The default settings were kept (number of voices 12, vanishing moment 20, threshold 40, oscillation width threshold 1.4, oscillation frequency spread threshold 10, spike width threshold 1.3, spike frequency spread threshold 11). If the peaks of IEDs and HFOs co-occurred within 100 ms, the IED was classified as an 'HFO-IED'[64]. The remaining IEDs were grouped as 'non-HFO-IEDs'. For the hippocampal recordings, the automatically detected rates of HFO-IEDs correlated significantly with visual identification ($p < 0.05$, Pearson correlation).

HFO-IEDs and the seizure onset zone (SOZ)

First, we applied the Delphos detector to investigate whether HFO-IEDs were specifically associated with the seizure onset zone (SOZ). The SOZ was defined by contacts with a clearly ictal EEG pattern within two seconds after seizure onset. All remaining contacts were classified as non-SOZ contacts. Contacts located in white matter were excluded from analysis. Rates of HFO- and non-HFO-IEDs were determined for the 30-minute interval that we had selected for our main analyses. Pooling channels across subjects, we found that HFO-IED rates were significantly higher inside the SOZ ($p < 0.001$, Wilcoxon rank sum test; SOZ: $n = 108$ channels, non-SOZ: $n = 386$ channels; Suppl. Fig. 4A). HFO-IED rates were also significantly higher if we compared them at the subject

level, i.e. each patient's median SOZ- and non-SOZ channel ($p < 0.05$, Wilcoxon signed-ranks test). Moreover, the percentage of IEDs carrying an HFO (i.e. HFO-IEDs / (HFO-IEDs + non-HFO-IEDs)) was significantly higher inside the SOZ - again regardless of whether we pooled channels across patients ($p < 0.001$; Suppl. Fig. 4B) or compared at the subject level ($p < 0.05$).

HFO-IEDs prior to seizures

Next, we aimed to investigate whether HFO-IEDs are associated with seizures. We therefore selected a maximum of three seizures from each subject, aiming to minimize the risk that group results predominantly reflected an effect in a few patients with many seizures. Purely electrographic seizures and seizures that arose less than one hour after a previous seizure were not considered. If more than three seizures fulfilled inclusion criteria, we selected those recorded at the end of the implantation period. This was done to minimize the possibly confounding effects of tissue irritation related to surgery and of antiepileptic medication, which had been tapered during the evaluation period in some patients. We then applied the Delphos detector on the recordings from the anterior hippocampus (i.e., the channels selected for our main analyses) to determine the percentage of HFO-IEDs in two 5-minute intervals: One interval immediately prior to seizure onset ('pre-ictal') and one interval 20 minutes before ('interictal'). We found that the percentage of HFO-IEDs was significantly higher in the pre-ictal interval ($p < 0.01$, Wilcoxon signed-ranks test; $n = 25$ seizures). This suggests that specifically HFO-IEDs may be associated with seizures.

HFO-IEDs and propagation

It might be hypothesized that HFO-IEDs are more pronounced epileptic discharges than non-HFO-IEDs, generated by larger networks and involving multiple brain regions. For each of our visually identified IEDs, we therefore examined if there was an associated IED in a non-hippocampal ipsilateral channel (within a window of ± 200 ms). IEDs in these non-hippocampal channels had again been detected by the Delphos algorithm. If a co-occurring IED was found, the visually identified IED was labeled as an 'IED with propagation'; if not, it was categorized as an 'isolated IED'. Comparing HFO- and non-HFO-IEDs, we found that 62 % of the HFO-IEDs were grouped as such IEDs with propagation, which was significantly more often than in non-HFO-IEDs (49 %; $p < 0.001$, Fisher's exact test).

Neuronal firing during ripple- and fast ripple-IEDs

Previous work suggests that the neuronal mechanisms underlying ripples and fast ripple are different (see e.g.[53] for a review). We therefore examined neuronal firing during IEDs with a co-occurring ripple oscillation ('ripple-IEDs') and during IEDs with a fast ripple ('fast ripple-IEDs'). Statistical analysis was again based on comparison to surrogate data, analogous to Figures 2D-F. In both ripple- and fast ripple-IEDs, we observed pronounced and significant increases in firing, which tended to be slightly higher in fast ripple ($p < 0.001$, cluster-based surrogate test, -140 to +20 ms; n = 108 multi units; Suppl. Fig 6A) than in ripple-IEDs ($p < 0.001$, -160 to +20 ms; Suppl. Fig. 6B).

Neuronal firing during ripples with and without IED

Especially ripples occurring independent from IEDs are also generated physiologically. Here, we therefore aimed to examine if, during such ripples without a clear IED, there was an increase in neuronal firing, similar to the increase in firing found in ripples with IED ('IED-ripples'). To this end, ripples without a clear IED were marked in the macroelectrode recordings that had been chosen for our main analyses. Thus, we were able to compare visually identified ripples with IED (marked for our main analyses) to visually identified ripples without IED. Events were aligned to the maximum amplitude of the ripple oscillation. Statistical analysis was based on a matched number of events, with a comparison to surrogate data analogous to Figure 2F. Direct comparison of ripples with and without IED revealed that the increase in neuronal firing was significantly higher in ripples with IED ($p < 0.001$, cluster-based permutation test, -70 ms to +10 ms; Suppl. Fig. 7).

Intense neuronal firing and spike sorting

It has been reported that intense neuronal firing during seizures alters the shape of neuronal spikes, and that this can systematically confound spike sorting[65]. Given that we also observed pronounced increases in firing, especially during the HFO component of HFO-IEDs, this could have been a potentially confounding effect in our study. To examine whether such an effect had a significant impact on our results, we performed the following analysis: Each spike was assigned to one of two groups, either group 'baseline spike' (if the spike did not occur during the HFO component of IEDs) or group 'HFO spike' (if the spike occurred during this HFO component). For each single unit, we first compared each baseline spike shape to all other baseline spike shapes of this unit, calculated a Pearson correlation coefficient r for each of these comparisons, and determined the median of all

these r's ('baseline median r'). An analogous procedure was then performed for the baseline vs. HFO comparison in this unit, i.e. we compared each baseline spike shape to all HFO spike shapes, calculated r each of these times, and determined their median ('baseline vs. HFO median r'). Finally, comparing the baseline median r's and the baseline vs. HFO median r's from our units, we found that there was no significant difference ($p = 0.76$, Wilcoxon signed-ranks test; $n = 40$ single units). This suggests that the spike shape of HFO spikes was not systematically different from baseline spikes.