Neuronal Firing and Waveform Alterations through Ictal Recruitment in Humans

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Analyzing neuronal activity during human seizures is pivotal to understanding mechanisms of seizure onset and propagation. These analyses, however, invariably using extracellular recordings, are greatly hindered by various phenomena that are well established in animal studies: changes in local ionic concentration, changes in ionic conductance, and intense, hypersynchronous firing. The first two alter the action potential waveform, whereas the third increases the “noise”; all three factors confound attempts to detect and classify single neurons. To address these analytical difficulties, we developed a novel template-matching-based spike sorting method, which enabled identification of 1239 single neurons in 27 patients (13 female) with intractable focal epilepsy, that were tracked throughout multiple seizures. These new analyses showed continued neuronal firing with widespread intense activation and stereotyped action potential alterations in tissue that was invaded by the seizure: neurons displayed increased waveform duration ($p < 0.001$) and reduced amplitude ($p < 0.001$), consistent with prior animal studies. By contrast, neurons in “penumbral” regions (those receiving intense local synaptic drive from the seizure but without neuronal evidence of local seizure invasion) showed stable waveforms. All neurons returned to their preictal waveforms after seizure termination. We conclude that the distinction between “core” territories invaded by the seizure versus “penumbral” territories is evident at the level of single neurons. Furthermore, the increased waveform duration and decreased waveform amplitude are neuron-intrinsic hallmarks of seizure invasion that impede traditional spike sorting and could be used as defining characteristics of local recruitment.

Key words: action potential; epilepsy; human; seizure; single neuron; single unit

Significance Statement

Animal studies consistently show marked changes in action potential waveform during epileptic discharges, but acquiring similar evidence in humans has proven difficult. Assessing neuronal involvement in ictal events is pivotal to understanding seizure dynamics and in defining clinical localization of epileptic pathology. Using a novel method to track neuronal firing, we analyzed microelectrode array recordings of spontaneously occurring human seizures, and here report two dichotomous activity patterns. In cortex that is recruited to the seizure, neuronal firing rates increase and waveforms become longer in duration and shorter in amplitude as the neurons are recruited to the seizure, while penumbral tissue shows stable action potentials, in keeping with the “dual territory” model of seizure dynamics.

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Introduction

A complete understanding of the mechanisms underlying seizure pathology and dynamics depends on knowledge of the local neuronal activity and what is driving that activity. Comparative animal models have long been used to gain insights into the underlying neuronal activity during seizures (Purpura et al., 1972; Fariello et al., 1976; Grone and Baraban, 2015), with the paroxysmal depolarizing shift (PDS), for more than half a century, being regarded as the intracellular correlate of ictal discharges in animal models (Kandel and Spencer, 1961a,b; Matsumoto and Marsan, 1964; Traub and Wong, 1982).

More recently, early PDSs have been shown to evolve into seizures *in vivo* (Steriade and Amzica, 1999), and PDSs have been recorded in resected human cortical tissue (Marcuccilli et al., 2010; Eissa et al., 2016). The PDS causes a decrease in action potential amplitude and an increase in half-width, features that should impede standard spike sorting methods, and yet this phenomenon has not been reported in several studies of single-unit activity during spontaneous human seizures (Wyler et al., 1982; Babb et al., 1987; Stead et al., 2010; Truccolo et al., 2011, 2014; Bower et al., 2012). Indeed, beyond the PDS, altered action potential waveforms could be expected following seizure invasion of the recording site, which we refer to as “recruitment.” Because of the intense bursts of neural firing, alterations to Na^+ and K^+ concentrations are present in the intracellular and extracellular space, which impact action potential wave shapes (Harris et al., 2000).

We have shown preliminary evidence of such potential alterations (Merricks et al., 2015). In tissue recruited to the seizure, traditional spike sorting methods can fail to cluster single units in human ictal recordings from neocortical layers 4/5, where neuronal cell body density is particularly high (Keller et al., 2018), thereby hindering the ability to track evidence of wave shape alterations or neuronal firing patterns during and after ictal recruitment, with zero clusters surviving detection during the seizure (Merricks et al., 2015; Fig. 1). However, whether this originated from alterations to neurons’ intrinsic action potential shapes or simply from destructive interference of waveforms from nearby, highly active cells recorded on the same electrode has been unclear.

Here, we present analyses of neuronal activity in the human brain during focal seizures using novel template matching methods to characterize action potential waveform alterations and single-unit firing patterns, as the seizure approaches, recruits, and passes through the local cortical territory. We hypothesize that, similar to observations in animal models, human focal seizures consistently display alteration of intrinsic action potential shapes on ictal recruitment. Meanwhile, we have shown previously that unrecruited tissue can show the characteristic, rhythmic EEG activity of seizures because of intense, local synaptic activity, despite being outside the recruited tissue because of maintained feedforward inhibition: a situation we define as “penumbral” tissue (Schevon et al., 2012). We hypothesize that the distinction between recruited and penumbral tissue is maintained at the level of single neurons, with recruited cells displaying reduced waveform amplitude, and increased duration, an effect that is absent in penumbral sites demonstrating increased firing rates, but lacking typical seizure hallmarks.

Materials and Methods

Human recordings. Adult patients (13 female, 14 male) undergoing surgical evaluation for pharmaco-resistant focal epilepsy at Columbia University Irving Medical Center and University of Utah were implanted with either a 96-channel, 4×4 mm “Utah”-style microelectrode array (UMA; Blackrock Microsystems) or Behnke-Fried style microwires (BF array; Ad-tech Medical Equipment) simultaneous to standard clinical electrocorticography (ECoG) or stereo-electroencephalography, respectively. UMAs were implanted into neocortical gyri based on presurgical estimation of the ictogenic region, with electrode tips reaching layer 4/5 (1.0 mm electrode length; layer confirmed via histology in Schevon et al., 2012), while BF arrays consisted of 8 microwires protruding ~ 4 mm from the tips of clinical depth electrodes (Misra et al., 2014).

Neural data were recorded at a sampling rate of 30 kHz on each microelectrode with a range of ± 8 mV at 16-bit precision, with a 0.3 Hz

Figure 1. Effects of ictal recruitment on traditional spike sorting methods. Spike sorting relies on stable waveforms from nearby neurons, but ictal activity disrupts features used to cluster single units. **A**, Example broadband local field potential (LFP) from a single channel of a UMA implanted in the posterior temporal lobe of a patient with pharmaco-resistant epilepsy (Patient 4, seizure 1). Dashed red line indicates “global” seizure onset. **B**, Bandpass filtered signal between 300 Hz and 5 kHz of the same signal in **A**, showing stable single-unit activity in the preictal period, and characteristic “tonic” firing (asterisk), followed by bursting. **C**, First principal component score versus time (**Ci**) of all detected waveforms in the MUA shown in **B**, with three clearly separable clusters highlighted, along with the multiunit cluster from background distal cells (black). **Cii**, Equivalent first versus second principal component scores from the preictal period showing well-isolated clusters, and (**Ciii**), during the seizure, showing loss of well-defined clusters in principal component space and inability to spike sort using traditional methods.

to 7.5 kHz bandpass filter on a Neuroport Neural Monitoring System (Blackrock Microsystems). Electrocorticography data from subdural or depth arrays were collected with a sampling rate of either 500 Hz or 2 kHz, with 24-bit precision and a bandpass filter of 0.5 Hz to one-fourth the sampling rate. In UMAs, the reference was either subdural or epidural, chosen based on recording quality. In BF arrays, the reference was the ninth microwire within the bundle.

All procedures were approved by the Institutional Review Boards of Columbia University Irving Medical Center and University of Utah, and all patients provided informed consent before surgery, as described previously. Clinical determination of seizure onset zone (SOZ) and seizure spread was made initially by the treating physicians and confirmed before analysis by two board-certified neurologists (C.A.S. and L.M.B.). BF arrays were localized using Brainlab (Brainlab).

Statistical analyses. All analyses were performed offline using custom scripts and toolboxes written in MATLAB (The MathWorks). Code is available at <https://github.com/edmerix>. All statistical tests for significance were performed using the Mann–Whitney *U* test unless otherwise noted, because of the non-Gaussian distributions of data requiring non-parametric testing. For all tests, the level for statistical significance (α)

Table 1. Demographics and data for patients implanted with UMAs^a

Patient	Demographics	Pathology	Implant location	Seizure type	Seizure onset	Seizures analyzed	No. of units
1	30 M	NA (multiple subpial transections performed)	Frontal (left frontal convexity)	FIA	Left supplementary motor area	1	79
2	39 M	Nonspecific	Frontal (left dorsolateral frontal lobe)	FIA	Left frontal operculum	2	61, 61
3	25 F	Mild CA1 neuronal loss; lateral temporal nonspecific	Temporal (left inferior temporal gyrus)	FIA	Left basal/anterior temporal	3	111, 101, 91
4	19 F	Nonspecific	Temporal (right posterior temporal)	FTBTC	Right posterior lateral temporal	1	110
5	24 M	Mesial temporal sclerosis; nonspecific	Temporal (left inferior temporal gyrus)	FIA	Left mesial temporal with spread to lateral temporal	2	120, 133
6	30 M	Mild astrocytosis	Temporal (right mesial temporal gyrus)	FA	Right subtemporal	1	240

^aIndividual patient demographics for grid/UMA implant cases, including the number of isolated single units found for each seizure in each patient. FIA, Focal with impaired awareness; FTBTC, focal to bilateral tonic-clonic.

Table 2. Demographics and results for patients implanted with BF arrays^a

Patient no.	Demographics	Pathology	Implant locations with units	Seizure type	Seizure onset	Seizures analyzed	No. of units	Microwires with waveform alterations	Time of recruitment (s)	Clinical correlation
7	29 F	Left frontal fibrotic calcified nodules	Mesial temporal (left hippocampus)	FTBTC	Left lateral frontal	2	2, 2	Left hippocampus	16.3	Spread
8	29 F	NA	Mesial temporal (left hippocampus)	FA	Left mesial temporal	2	2, 1	Left hippocampal head	16.1	√/+
9	55 F	FCD 1B/1C; Chaslin's gliosis; hippocampal sclerosis	Mesial temporal (right hippocampus)	FIA	Right lateral temporal	1	9	—	—	√/—
10	25 M	NA	Mesial temporal (left hippocampal body)	FIA	Right mesial temporal and right parietal (dual)	1	1	Left hippocampal body	42.7	Spread
11	19 M	FCD 1B/1C; Chaslin's gliosis; reactive gliosis	Frontal (right anterior cingulate)	FIA	Right lateral temporal	1	3	Right supplementary motor area	9.1	Spread
12	20 F	NA	Frontal (left anterior and mid-cingulate)	FA	Left mesial temporal	1	15	Left mid-cingulate	19.5	Spread
13	21 F	Astroglia and microglia	Mesial temporal (left hippocampus)	FA	Left anterior temporal pole	1	1	—	—	√/—
14	40 M	FCD 2B	Mesial temporal/frontal (left hippocampus and anterior cingulate)	FIA	Left lateral frontal	2	9, 10	Left hippocampus; Left mid-cingulate	39.3 (HC); 29.0 (MC)	Spread
15	25 F	Astroglia and microglia	Mesial temporal (right hippocampus body and head)	FIA	Right lateral temporal spreading to mesial temporal	1	11	Right hippocampal body; Right hippocampal head	9.1 (HCB); 8.0 (HCH)	√/+
16	35 F	NA	Mesial temporal (bilateral hippocampus)	FIA	Left mesial temporal	1	5	Left hippocampal head	8.0	√/+
17	40 M	NA	Mesial temporal (left hippocampus)	FIA	Left mesial parietal	1	2	—	—	√/—
18	39 M	NA	Frontal (right mesial cingulate)	FIA; FTBTC	Right mesial temporal and left orbitofrontal (2 types)	2	8, 4	—	—	√/—
19	30 M	NA	Frontal (right mesial cingulate)	FA; FIA	Left mesial temporal and left insula	2	1, 1	—	—	√/—
20	23 F	FCD 2B	Mesial temporal (right hippocampus)	FIA	Right posterior temporal	1	3	—	—	√/—
21	35 M	NA	Mesial temporal (bilateral hippocampus)	FA	Right insular and somatosensory cortex	3	7, 8, 7	Right hippocampal head	Subthreshold	Spread / × +
22	44 F	Left frontal reactive astrocytosis; Chaslin's gliosis in mid-cingulate	Frontal (right anterior cingulate)	FTBTC	Left cingulate	1	1	Right anterior/mid-cingulate	15.2	Spread
23	51 F	NA	Frontal (left anterior cingulate)	FIA	Left mesial temporal	1	4	Left anterior cingulate	12.6	Spread
24	20 M	NA	Frontal (left anterior cingulate)	FIA	Left posterior temporal	1	1	—	—	√/—
25	30 F	Reactive astrocytosis and microglia; leptomeningeal inflammation and focal cavitation	Frontal (right anterior cingulate)	FIA	Left mesial temporal	1	1	—	—	√/—
26	20 M	Ganglioglioma (WHO Grade 1)	Mesial temporal (right hippocampus)	FIA	Right mesial temporal	2	1, 2	Right hippocampal body	10.4	√/+
27	32 M	NA	Mesial temporal (bilateral hippocampus)	FIA	Bilateral mesial temporal	3	4, 3, 3	Hippocampal body	9.8	√/+

^aIndividual patient demographics for stereo-EEG/BF array cases, including the number of isolated single units found for each seizure in each patient, implant locations that showed significant wave shape alterations, the delay until waveforms surpassed >1 SD beyond the preictal mean, and whether clinical observations matched these findings. FTBTC, Focal to bilateral tonic-clonic; FA, focal aware; FIA, focal with impaired awareness; HC, hippocampus; MC, mid-cingulate; "Spread," matches clinical observations of seizure spread; √+, true positive match for clinical observations; √/—, true negative match for clinical observations; ×+, false positive match for clinical observations; FCD, focal cortical dysplasia; pathology NA in some cases that were not resected, or that were treated with laser ablation or an implanted device.

was set to 0.05, and Holm-Bonferroni correction was applied in all instances of multiple tests.

Local recruitment to the seizure. We have previously proposed that focal seizures are comprised of two coexisting neuronal behavior patterns in regions that exhibit abnormal EEG activity during the seizure, characterizing distinct territories that are unique to each individual. Full ictal recruitment features aberrant local firing and synaptic activity. "Penumbra" activity is characterized by rhythmic EEG activity because of intense synaptic barrage from the seizure, but heterogeneous neural firing (Schevon et al., 2012, 2019). Local ictal recruitment occurs when the leading edge of the seizure activity (the "ictal wavefront") successfully

propagates into the local tissue (Trevelyan et al., 2006, 2007; Schevon et al., 2012; Liou et al., 2020). The hallmark of this ictal wavefront is a transient "tonic" firing pattern, characterized by a high firing rate and lack of oscillatory organization (Fig. 1B, asterisk). This event defines recruitment to the seizure, while the "burst" firing that follows is the extracellular correlate of the PDS across a neuronal population that has been recruited to the seizure (Matsumoto and Marsan, 1964; Tryba et al., 2019; Liou et al., 2020).

Unlike the SOZ, which is the region of earliest ictal activity, ictal recruitment and the penumbra are spatially and temporally dynamic, as the seizure wavefront propagates through brain tissue (Smith et al.,

Figure 2. Template match spike sorting via convex hulls. **A**, Well-isolated neurons form distinct clusters in principal component space during interictal time points (**Ai**), around which a convex hull can be fitted to define boundaries in 3D space within which waveforms that match that unit should exist (**Aii**). Despite a lack of defined clusters during ictal activity in recruited cortex, this convex hull can be used to select waveforms that are likely to correspond to the preictal unit (**Aiii**; black), among distributed noise (gray). **Bi**, Waveforms matched using the convex hull shown in **Aiii** (black) from the large distribution of waveforms during a seizure (gray). **C**, Outliers in the χ^2 distribution for n degrees of freedom (red line) denote likely incorrect matches, and were automatically removed to minimize false positives (see Materials and Methods).

2016). As such, a fixed location, unless at the true origin of the initial ictal activity, may receive the synaptic input of the upstream seizure but remain unrecruited (“penumbral”) initially, before the transition to the ictal state which may occur at any point during the seizure.

UMAs detect relatively large numbers of single neurons from nearby space, allowing for analysis of multiunit activity (MUA) and its spatial organization across the sampled territory (Table 1). Therefore, the timing of the passage of the ictal wavefront at individual electrodes was calculated based on the MUA firing rate in UMAs. A Gaussian kernel of 500 ms duration was convolved with the timings of all detected waveforms in the MUA, and a sustained, significant increase in the resultant instantaneous firing rate was determined as the moment of local recruitment to the seizure as previously defined (Smith et al., 2016). Ictal recordings without this signature of tonic to burst MUA firing were classified as penumbral.

BF arrays, meanwhile, record from relatively few disparately located neurons (Table 2); thus, MUA firing cannot be readily detected for use in defining ictal recruitment (see Discussion). Therefore, we used these cases instead as a robustness test of UMA population findings relative to clinical information, making use of the spatial distribution of BF array implants.

Preliminary single-unit discrimination. Initial spike sorting was performed on the period before and after the seizure (“peri-ictal”) as per Merricks et al. (2015). Briefly, neural signals were symmetrically band-pass filtered between 300 Hz and 5 kHz (1000th order FIR1) to extract MUA, from which extracellular action potential waveforms were detected using a voltage threshold of 4.5σ , where $\sigma = \frac{\text{median}[x]}{0.6745}$ and x is the MUA from that channel. This method avoids the biasing effect of large waveforms on channels with units with high firing rates (Quian Quiroga et al., 2004). Ictal periods were blanked so that spike

sorting was only performed on stable waveforms from the peri-ictal period.

Matrices of waveforms from each channel were created from 0.6 ms before 1 ms after each detection, and principal component-based semi-automatic cluster cutting was performed using a modified version of the “UltraMegaSort2000” MATLAB toolbox (Fee et al., 1996; Hill et al., 2011). We devised a novel method for removing nonphysiological artifacts by calculating the FFT on all detected spikes in the MUA, and removing those with power >5 SDs above the mean in frequencies >2.5 kHz or <500 Hz. While these frequencies are found in physiological signals, higher power in these bands corresponded to electrical and/or motion artifact, being faster and slower than the main components of extracellular action potentials, respectively, with a complete period of <0.4 ms or >2 ms. Spikes removed in this manner were visually inspected to ensure correct classification as artifact (2.06% total, $n = 3317$ of 160,887 spikes). Clusters that satisfied the following criteria were accepted as follows: (1) clean separation from all other clusters in the Fisher’s linear discriminant in principal component space; (2) $<1\%$ contamination of the 2 ms absolute refractory period; (3) no clear outliers based on the anticipated χ^2 distribution of Mahalanobis distances; and (4) $<1\%$ of estimated false negatives as estimated by the amount of a Gaussian fit to the detected voltages fell below the threshold for detection, as described in Hill et al. (2011).

Template matching through seizures. We developed novel methods to match waveforms from the ictal period, regardless of recruitment, to their putative neuronal source based on templates derived from the peri-ictal units. In contrast to standard spike sorting methods, our aim with these methods was to minimize false negatives at the expense of increasing a limited number of false positives so as to avoid missing potential matches and make it possible to distinguish between neurons ceasing

firing as opposed to becoming untrackable by the method. To template match waveforms, principal components were calculated separately for each channel, on a matrix of all waveforms from the previously defined units from both the preictal and postictal period recorded at that electrode. Cluster boundaries were then defined as the 3D convex hull surrounding the scores for the three principal components that contained the largest variance of the original data for that channel, using the MATLAB function “convhull” (Fig. 2). The first three components were chosen as convhull functions in up to 3D space; and while “convhulln” could be used to increase specificity at the expense of sensitivity in future analyses, here we wished to avoid potential false negatives from a more restricted approach, which would result from using higher dimensions. Using only the first three components accounted for $96.5 \pm 1.82\%$ (mean $\pm$ SD) of the total variance in all original units, and $93.0 \pm 2.95\%$ of the variance in the matched waveforms.

This method accounts for two situations: that neurons within recruited cortex maintain their wave shape but are obscured by interference from other nearby cells; or that there are occasional or consistent alterations to a neuron’s intrinsic waveform that are minor enough to be maintained within the convex hull of feature space. The convex hull allows for alterations to wave shape in any dimension (any direction away from the cluster’s centroid).

To avoid ictal results being biased through differing methods, template matching was performed on waveforms that were extracted from a period from 10 min before 10 min after seizures, including the ictal activity that had been blanked in the original peri-ictal spike sorting. Channels with unstable units during interictal periods were excluded. Units with no waveforms in either the preictal or ictal time period after template matching and artifact removal were excluded from further analyses ($n = 77$), and units with mean firing rates of <0.1 spikes s^{-1} during the seizure were treated as being “untracked.”

Principal component scores were calculated on these waveforms based on the previously defined principal components, and waveforms that occurred within a peri-ictal unit’s convex hull were assigned to that cell. Mahalanobis distances were calculated for all matches, between their location in principal component space and all peri-ictal waveforms from that unit, on the first n principal components that explained $>95\%$ of the variance in the data set (Fig. 2C). The expected distribution of Mahalanobis distances was calculated as the χ^2 probability distribution with n degrees of freedom. Waveforms that had $<0.1\%$ chance of occurring in the χ^2 distribution were excluded.

Waveform metrics. For analyses of waveform shape after template matching, the FWHM and amplitude were used. The FWHM was calculated by interpolating each waveform by a factor of 4 to avoid quantization, and calculating the waveform’s duration at half its amplitude. When calculating waveform amplitude changes through the ictal transition, only units whose mean voltage at detection was at least 2.5 SDs away from that channel’s threshold for detection were used, to minimize the floor effect from small units that dropped below threshold.

Timing of FWHM alteration relative to the earliest ictal activity was determined by the earliest time point during the seizure that the mean FWHM remained above the preictal mean plus the preictal SD for at least 1 s, calculated in a sliding window of 5 s duration with a time step of 50 ms, discarding windows with <5 waveforms.

For analyses involving the probability that each waveform arose from its assigned peri-ictal unit, match confidences were calculated by fitting a separate Gaussian curve (with a maximum amplitude of 1) to

Figure 3. Waveform match probabilities. **Ai**, All waveforms from a traditionally spike sorted unit in a 10 min preictal epoch. The raw probability distribution of each voltage at each time point for these waveforms is shown in **Aii**, and the scaled waveform match confidence for ictal waveforms matching this unit are shown in **Aiii**, with the distribution of these probabilities inset. Waveform match confidences were calculated with each time/voltage probability scaled to have a maximum value of 1, such that a waveform going through the peak of each data point’s fitted Gaussian would have a match probability of 1. **B**, Top, The probability distribution of all matched waveforms (red), compared with the probability distribution for the original waveforms in the preictal time point (orange) for an example template-matched single unit. A bootstrap estimate of waveform matches expected by chance by comparing against waveforms from other electrodes is shown in blue. Bottom, The equivalent distributions across the full population. Mean $\pm$ SD is shown above the distributions.

the distribution of voltages at each data point in the original unit, and calculating the mean probability across all time samples. As such, a waveform passing through the most likely voltage at each separate time point for that unit would have a match probability of 1 (Fig. 3).

Firing rate calculations. Instantaneous firing rates were calculated by convolving the unit firing times with a Gaussian kernel (200 ms SD) with the amplitude scaled to each waveform’s probability of matching the original unit, thereby creating probabilistic firing rates for each unit through time, by probability of when the action potential occurred and probability that the waveform originated from the putative neuronal source. As such, a waveform that had an average probability of 20% across all fitted Gaussians from each data point would contribute only 0.2 spikes s^{-1} at its most likely time point, while an exact match to the most likely voltage at each time point would contribute 1 spike s^{-1} . Weighting the firing rates in this manner served to reduce the impact of false positives from the necessarily inclusive spike sorting methods. Thresholds for significant increases and decreases in firing rate were calculated as 3 times the square root of the firing rate divided by the duration of the epoch, which approximates 3 SDs for a Poisson distribution and allows for analysis of significant changes in firing rates of spike trains (Vajda et al., 2008).

Results

We analyzed ictal recordings from 27 patients undergoing invasive EEG monitoring as part of the presurgical evaluation for intractable focal epilepsy (Tables 1 and 2; age range = 19–55 years; 13 female, 14 male). Six patients were implanted with Utah microelectrode arrays (UMA; Blackrock Microsystems), and the remaining 21 patients were implanted with between 1 and 4 BF depth arrays with incorporated microwire bundles (BF arrays; Ad-tech Medical Equipment). A total of 41 seizures were

Table 3. Single-unit data from UMA population analyses^a

Patient no.	Seizure no.	Recruited?	Units from convex hull [n]	Units showing FR increase in ictal wavefront [n (%)]	Units showing significant FWHM increase [n (%)]	Units showing significant amplitude decrease [n (%)]
1	1	×	52	NA	2 (4)	3 (6)
2	1	×	55	NA	9 (16)	8 (15)
	2	×	49	NA	3 (6)	2 (4)
3	1	✓	80	77 (96)	63 (79)	20 (25)
	2	✓	80	70 (88)	68 (85)	38 (48)
	3	✓	79	67 (85)	66 (84)	40 (51)
4	1	✓	101	77 (76)	93 (92)	45 (45)
5	1	✓	117	82 (70)	114 (97)	103 (88)
	2	✓	104	73 (70)	85 (82)	63 (61)
6	1	×	221	NA	105 (48)	86 (39)

^aTotal number of units found in each patient and seizure for population analyses in UMA cases via the convex hull template-matching method, along with the number of units showing significant increases in firing rate, waveform FWHM, and decreases in amplitude during the seizure.

Figure 4. Neuronal firing through ictal recruitment in an example focal seizure. **A**, Local field potential (LFP) from an example channel during seizure 1 in Patient 3, with the calculated passage of ictal wavefront marked by red dashed line, with ± 2 s shaded red. **B**, Raster plot of all units found using convex hull template matching in this seizure, ordered by firing rate, and plotted relative to the moment of ictal recruitment at that channel. **C**, Weighted (purple) and unadjusted (blue) instantaneous firing rate of the population of single units as estimated by convolving a Gaussian kernel (200 ms SD) over the spike times. The firing rate shown in purple is probabilistic by scaling the Gaussian kernel's amplitude by the likelihood of each individual waveform originating from its assigned preictal unit, as calculated by its voltage-time probabilities (see Materials and Methods; Fig. 3). As such, the firing rate has not been biased by excessive matching of dissimilar waveforms during the ictal activity. Note the intense, tonic firing during the seizure invasion, and sustained, above baseline firing until seizure termination.

reviewed (10 UMA; 31 BF array), of which 27 demonstrated ictal recruitment through MUA firing rate calculation in UMAs, or subsequent waveform alterations in BF arrays (see Materials and Methods; UMA: 6 seizures from three patients; BF arrays: 21 seizures from 13 patients).

Tracking neurons through seizures

To assess the presence of physiological waveform shape changes across populations of neurons, we used template matching using convex hulls (Fig. 2; see Materials and Methods) on each seizure. This allows for assigning unit identities without the need for spatially separated cluster boundaries in the data required for

standard spike sorting, while accepting waveform alterations that may occur as a consequence of the intense firing that accompanies seizure invasion. In total, 1239 units were isolated from the preictal and postictal period using traditional spike sorting, with the ictal time period blanked as described in Preliminary single-unit discrimination. Of these units derived from traditional spike sorting, 938 of the 1107 units recorded on UMAs and 120 of 132 units on BF arrays were tracked through seizures using this method. This is in contrast to previous abilities to spike sort through seizures in similar layer 4/5 recordings, where the requirement for stable clusters resulted in a retention rate of 37.7% across all recordings, and 0% in recruited tissue (Merricks et al., 2015). The 14.6% of “lost” units in the template-matched data here may have arisen from either cessation of firing or deformation of wave shape beyond the defined convex hull for that unit; we therefore analyzed the rate of untracked neurons with reference to whether there was MUA evidence of seizure invasion, defined as the arrival of the ictal wavefront (limited to UMA recordings; see Materials and Methods). While units were lost at similar rates regardless of location (mean $\pm$ SD lost in recruited vs penumbral recordings: $15.8 \pm 9.5\%$ vs $14.5 \pm 12.0\%$), the stage of analysis during which the units were lost differed by location. In recordings from recruited tissue, 104 of 105 (99.0%) neurons that were untracked were rejected because of too low firing rate in the preictal period to build templates, while 1 (0.95%) neuron was untracked because of too low firing rate during the seizure (<0.1 spikes s^{-1}). In contrast, in penumbral recordings, 37 of 64 (57.8%) neurons were untracked because of too low firing rate in the preictal period to build templates and the remaining 27 (42.2%) were rejected because of too low firing rates during the seizure, highlighting the role of quiescence in penumbral recordings but not in recruited tissue.

As the focus of this study was maximizing unit detection despite waveform deformations, our method was designed to maximize the likelihood of detecting ictal activity of the identified units. However, accepting all waveforms that fell within the

convex hull of a previously defined unit's principal component space increases the probability of false detections. With no ground truth, we therefore weighted each waveform's probability of being a match to their assigned unit by their waveform similarities (Fig. 3; see Waveform metrics). To assess the effect of this probabilistic method, we tested it on the preictal epochs in the population data from UMA recordings. Without the probabilistic weighting, the method substantially overestimated preictal firing rates (original vs matched mean $\pm$ SD: 0.436 ± 0.723 vs 0.798 ± 1.615 spikes s^{-1} ; $p < 0.001$). After weighting by waveform match confidence, the overestimation was greatly reduced (original vs matched mean $\pm$ SD: 0.436 ± 0.723 vs 0.487 ± 0.925 spikes s^{-1} ; $p = 0.695$).

Furthermore, waveform matches from the convex hull method were found to be more likely to arise from their assigned peri-ictal units than by chance, as calculated by the aforementioned waveform similarities. The "null" distribution for expected match probabilities by chance was calculated through comparing each waveform's similarity to the peri-ictal voltage-time distributions of all other units. Comparing intra-unit to inter-unit similarities in this way found a higher similarity between template-matched units and their presumed peri-ictal unit than to the "null" distribution of matches to other units ($p < 0.001$; Mann–Whitney U test; Fig. 3B).

While waveforms from other channels would plausibly appear in the same convex hull space as a given single unit because of the similarity in action potential morphology, we wished to ensure these higher similarities were not purely a result of more waveforms occurring outside the hull in other channels. To assess this, we compared the matched waveforms to all waveforms from other electrodes that occurred within the unit's convex hull. The matched data were found to have lower Mahalanobis distances within the convex hull and higher waveform match values than waveforms from other channels within the convex hull ($p < 0.0001$ in both cases).

Neuronal firing rates through the ictal transition

We analyzed the firing rates of template-matched unit populations throughout ictal activity in UMA recordings and related these to MUA evidence of seizure invasion, defined as the time of arrival of the ictal wavefront (Patients 3–5; see Materials and Methods). Single-unit firing rate increased following ictal recruitment in all seizures with 446 (79.5%) of 561 units showing >3 SD increase in firing rate, and only 1 unit in the entire population showing a >3 SD decrease in firing rate (range of

Figure 5. Example waveform changes at ictal recruitment in template-matched data. **A**, Waveforms from a convex hull-matched unit in Patient 5, seizure 2, showing reduction in amplitude and increase in FWHM during the seizure (red shaded waveforms, fading from black to red through the seizure; color maintained throughout figure). **B**, Waveform amplitude versus FWHM in the unit in **A**, showing equivalent relationship to the high amplitude BF single unit in Figure 9D. **C**, Wideband LFP from this channel through time (broken x axis), showing time of earliest ictal activity (blue triangle) and moment of local ictal recruitment (purple triangle), with **(D)** time-locked raster plots for original spike sorted unit (top) and convex hull matched data (bottom), showing high similarity in nonictal time points (peri-ictal firing rates: original = 2.63 spikes s^{-1} ; convex hull = 2.92 spikes s^{-1} ; the 25 s after recruitment is blanked in the original spike sorting because of a lack of distinguishable clusters). **E**, **F**, Time-locked FWHM and amplitude, respectively, showing temporal relationship of wave shape changes through the seizure. Note the return to preictal values after seizure termination, and the lack of changes toward decreased FWHM or increased amplitude during the seizure despite the convex hull being equally permissive of alterations in any direction.

single units with >3 SD increase per seizure: 70%–96%; see Table 3). An example seizure demonstrating these trends is shown in Figure 4, highlighting both the raw results from the convex hull matching (blue) and the weighted values to minimize the impact of false positives (purple).

To contextualize firing rates in seizures using these methods, we sought to gain further insight into the underlying signal-to-noise composition of single neuron recordings in recruited versus penumbral tissue. To do so, we analyzed the proportion of spike detections that were classified as action potentials from a single neuron in channels with only one single unit using the template-matched data. In recordings from recruited tissue, there was a large decrease in the signal-to-noise ratio, with $47.7 \pm 26.7\%$ of waveforms attributable to the single neuron in the preictal period, versus $11.9 \pm 8.1\%$ of waveforms during the

Figure 6. Stereotypy of waveform changes within neurons across seizures. Example waveforms from a single unit in Patient 5 showing stereotypy of response of extracellularly recorded action potentials during ictal recruitment in 2 seizures separated by 22 h (**A** and **B**, respectively). All waveforms from the 6 s before ictal recruitment are shown overlaid in **i** and plotted relative to time in **ii** (scales maintained throughout). Saturation of color fades from black at -6 s, to brightest at the moment of maximal firing rate of MUA at that electrode.

seizure ($p < 0.001$; Wilcoxon signed rank test, $n = 94$ neurons). In contrast, recordings from penumbral tissue were stable, with $44.4 \pm 27.3\%$ of waveforms being attributed to single neurons in the preictal period versus $36.8 \pm 26.7\%$ ($p = 0.09$; Wilcoxon signed rank test, $n = 33$ neurons). These values correspond to no significant difference between regions in the preictal period ($p = 0.4799$; Mann–Whitney U test) and a significant decrease in signal-to-noise ratio in single neuron recordings in recruited tissue compared with penumbral recordings ($p < 0.0001$; Mann–Whitney U test).

Dual effects of seizures on single-unit waveforms

Single-neuron studies in acute mouse seizure models show increases in action potential duration (Codadu et al., 2019) and decreases in action potential amplitude (compare Merricks et al., 2015, their Supplementary Fig. 4: 0 Mg^{2+} in vitro cell-attached mouse slice model). We therefore predicted that wave shapes in recruited tissue would change in one direction in each metric, so if the convex hull method worked, we should see not a random change in waveform shape, but a consistent finding of increased duration and decreased amplitude. Individually, results from the template matching method in recordings from recruited tissue (see Materials and Methods; Patients 3–5) showed decreases in waveform amplitude and increases in waveform FWHM (Fig. 5, ictal waveforms in red), and were stereotyped across seizures within patient (Fig. 6). At the population level, units in recruited cortex displayed a significant global increase in FWHM (Fig. 7A; preictal vs ictal mean $\pm$ SD: 0.470 ± 0.137 ms vs 0.611 ± 0.194 ms), with 457 (81.5%) of 561 single units showing a significant increase in FWHM during the seizure (Holm–Bonferroni-corrected Mann–Whitney U test; range across seizures: 79%–97%; see Table 3). Meanwhile, units in penumbral cortex (i.e., where no ictal wavefront was detected)

showed only a minor increase in FWHM at the population level (Fig. 7B; preictal vs ictal mean $\pm$ SD: 0.414 ± 0.009 ms vs 0.429 ± 0.099 ms) with only 9 (5.8%) of 156 single units showing a significant ($p < 0.05$) increase in FWHM during the seizure (Holm–Bonferroni-corrected Mann–Whitney U test; range across seizures: 4%–16%; see Table 3). In a single case (Patient 6), the UMA was at the edge of ictal spread based on clinical and EEG assessment; and in this patient, 105 (47.5%) of 221 units showed a significant increase in FWHM (preictal vs ictal mean $\pm$ SD: 0.408 ± 0.111 ms vs 0.421 ± 0.152 ms). These waveform shape alterations coexisted with stable wave shapes elsewhere on the UMA at the same time (Fig. 8), and this patient’s recordings were incorporated into the penumbral dataset for population representation in the figures (Fig. 7). FWHM increases in recruited tissue were significantly larger than those in penumbral/edge case recordings ($p < 0.001$; one-tailed Mann–Whitney U test).

Similarly, units in recruited cortex showed a decrease in waveform amplitude during the seizure (Fig. 7C; preictal vs ictal mean $\pm$ SD: $48.82 \pm 30.91 \mu\text{V}$ vs $34.96 \pm 19.54 \mu\text{V}$), while penumbral recordings maintained their peri-ictal amplitude (Fig. 7D; preictal vs ictal mean $\pm$ SD: $46.69 \pm 16.59 \mu\text{V}$ vs $45.08 \pm 15.05 \mu\text{V}$ in fully penumbral cases; $47.00 \pm 21.18 \mu\text{V}$ vs $45.57 \pm 20.93 \mu\text{V}$ in the semirecruited UMA). The amplitude reduction in recruited tissue was greater than in penumbral/edge case recordings ($p < 0.001$; one-tailed Mann–Whitney U test), with recruited recordings showing significant decreases in amplitude during the seizure in 49.3% of units vs 6.4% of units in the penumbra and 38.9% in semirecruited tissue (Holm–Bonferroni-corrected Mann–Whitney U test). The direction of these changes, a decrease in amplitude and an increase in FWHM, was consistent throughout the combined dataset of recruited cortex, and seen in all recruited seizures, with only 1 neuron in recruited cortex showing the inverse effect of a significant decrease in FWHM (0.18%) and 13 (2.32%) showing an increase in amplitude.

To confirm that these findings were not a result of the template matching method introducing an unknown variable that affected our waveform measurements, results from the original spike sorted data and those originating from the convex hull method on the preictal period were compared, finding little difference between the traditional cluster cutting results and the convex hull matched results (Fig. 7A–Di, inset, cumulative histograms).

To ensure that these results were not biased by outlier effects in any individual seizure, all analyses were subjected to a “leave-one-out” robustness test. In recruited cortex, the mean $\pm$ SD results for FWHM across all permutations while leaving one seizure out were 0.462 ± 0.0054 ms in the preictal period and 0.600 ± 0.013 ms in the ictal period, with $82.3 \pm 1.4\%$ of units showing significant changes. Similarly, the amplitude results were maintained with preictal values of $48.59 \pm 5.91 \mu\text{V}$ and ictal values of $35.06 \pm 2.61 \mu\text{V}$ across all permutations.

Similarly, we sought to confirm that outliers in the penumbral group were a result of the edge case recording in Patient 6. In total, 8 units were found to contribute >1 SD above the mean to the ictal distribution of short FWHMs (Fig. 7Bi), accounting for 66.6% of all waveforms in this range. Of these, no difference in

Figure 7. Population wave shape alterations in recruited cortex versus penumbral territories. **Ai, Bi**, Probability density plots of waveform FWHM for every detected waveform in the preictal (blue) and ictal time periods for all seizures in recruited cortex (**A**; $n = 136,421$ preictal waveforms vs 128,845 ictal waveforms from 625 units across 6 seizures in 3 patients) and penumbral tissue (**B**; $n = 133,424$ preictal waveforms vs 36,900 ictal waveforms from 405 units across 4 seizures in 3 patients). Note the shift in distribution in recruited tissue, while changes in penumbral tissue were minimal, remaining in the same footprint. An increase in firing rate in waveforms of brief FWHM from units that did not change shape resulted in an increase in the values in the left-tail of the penumbral data, originating from the edge-case and likely corresponding to fast-spiking interneuronal firing (see Dual effects of seizures on single-unit waveforms). Cumulative probability densities show same calculation on preictal, original data (black). **ii**, Paired mean FWHM for each unit in the preictal (blue), ictal (red), and postictal (pink) epochs. Note return to preictal ranges after seizure termination. **iii**, Waveform FWHM of the population through time (10 s window, sliding every 100 ms). Brighter indicates more density. Blue line indicates the mean through time. Purple line indicates the mean value in the preictal period. Red dashed line indicates “global” seizure onset. **C, D**, Same format as in **A** and **B**, showing waveform amplitude in place of FWHM, for recruited cortex and penumbra, respectively.

Figure 8. Simultaneous recruitment and penumbral recording. Two types of activity pattern recorded simultaneously in a patient with the UMA at the edge of the clinically defined seizure spread (Patient 6). **A**, LFP from the closest macro-electrode to the UMA, with time-locked MUA (blue) and first principal component score (purple) through time from channels 88 and 21 in **B** and **C**, respectively. Note the stability of waveform and principal component score throughout the seizure in channel 88, with no evidence of tonic firing, while there is a large wave shape change at the same time in channel 21, at the moment of tonic firing (maroon bar, **C**). **B**, **C**, Paired orange bars represent burst firing at the end of the seizure in both locations. These dual activity types both occurred immediately next to the LFP in **A**; Thus, these patterns cannot be differentiated at the macro LFP level.

FWHM was found between the preictal and ictal time points ($p = 0.35$), and all were found to increase their firing rate substantially during the seizure (mean increase in firing rate of 17.0 SD above preictal levels, range: 10.5–27.1 SD increase), confirming that this distribution shift was a result of increased firing in a subset of neurons, rather than waveform alterations. Furthermore, we found that 86.1% and 85.4% of significant changes in FWHM and amplitude, respectively, in the penumbral/edge case group were attributable to that single patient.

Action potential changes in deep structures

To assess the spatiotemporal relationship between waveform alterations and seizure recruitment within patients, beyond the capabilities of the 4 mm² UMA and in deep structures, such as the hippocampus, we analyzed BF array recordings with the equivalent template matching, blind to the clinically defined SOZ and areas of propagation. In BF array recordings, 30 of 120 single units (25.0%) showed increases beyond a cutoff significance level of $p < 0.05$ (Holm-Bonferroni-corrected Mann-Whitney U test) in FWHM (17 seizures from 13 patients), and 30 units (25.0%; 16 seizures from 11 patients) showed reduction in waveform amplitude below the same significance cutoff ($p < 0.05$; Table 2). In 9 seizures from 6 patients, single units were simultaneously present on multiple separate BF arrays (on different bundles of microwires, as opposed to different microwires within a single BF). Of these, 2 seizures (2 patients) showed significant waveform alterations in dual locations, indicative of recruitment in multiple locations (Patients 14 and 15; Fig. 9; Table 2), while 5 seizures (4 patients) showed both activity types simultaneously, indicative of recruitment in one location while a second recorded location remained outside the seizure-invaded region (Patients 11, 12, 16, and 21; Movie 1).

Five of the 13 patients with significant waveform alterations had a clinically defined SOZ in the mesial temporal lobe, and simultaneously recorded single units in the ipsilateral hippocampus demonstrated significant waveform alterations (Patients 8, 15, 16, 26, and 27; Table 2). In 7 further patients, significant waveform alterations were found in tissue consistent with putative seizure spread. In 1 case (Patient 10), we found waveform

alterations in the contralateral hippocampal body, consistent with clinical observations of contralateral seizure spread in this case. Conversely, in the 8 patients showing no significant waveform alterations, the clinically defined SOZ was anatomically distant in all cases (Patients 9, 13, 17–20, 24, and 25; Table 2).

In one instance of a patient recorded with BF arrays in the hippocampal head and body, a peculiarly discrete unit cluster because of a very large amplitude waveform (mean = 354 μ V; background noise level = 25 μ V) enabled us to follow its action potential through the ictal transition by visual inspection alone, despite marked changes to wave shape and increases in other unit activity (Fig. 9; Movie 2) (Schevon et al., 2019). In this example, the firing rate of the high amplitude unit increased abruptly ~ 8 s after seizure initiation (Patient 15; Table 2). Both the action potential amplitude and FWHM were stable before this time and preictally, but amplitude reduced and FWHM

increased sharply on the abrupt increase in firing rate (Fig. 9; preictal vs ictal mean $\pm$ SD amplitude and FWHM: 354.2 $\pm$ 45.7 μ V vs 265.7 $\pm$ 33.4 μ V and 0.33 $\pm$ 0.04 ms vs 0.40 $\pm$ 0.05 ms; both $p < 0.001$, Mann-Whitney U test).

A simultaneously recorded single unit on a separate BF microwire bundle located in the same hippocampus at ~ 1 cm away demonstrated the same changes in amplitude and FWHM developing at ~ 1 s later (Fig. 9Bii; Table 2). The distinct time course of these two physically disparate units shows that the alterations are unit-specific and are unlikely to be caused by physiological changes as a result of the seizure that affect large regions, or by movement artifacts (the seizure had a bland semiology, with no observed stress on the recording device). Together, these findings are suggestive of a slowly propagating seizure through the hippocampus, with waveform amplitude and FWHM timings echoing the template-matched results in the UMA (note the similarity between Fig. 5B and Fig. 9D), and occurring in tandem (Fig. 9D, blue through red transition, colors maintained from Fig. 9A).

Action potential changes in relation to seizure recruitment

To assess whether the classification of ictal recruitment via waveform alterations was consistent with the clinically defined SOZ and regions of spread, the time from earliest ictal activity to a consistent (≥ 1 s duration) increase in FWHM ≥ 1 SD above the mean preictal level for each unit was calculated. These analyses were split by recording type to assess different recruitment properties: BF array recordings allow for multiple spatial sampling, comparing waveform changes in different regions; while UMA recordings allow for analysis of the ictal wavefront propagation in a single location with many simultaneously recorded neurons.

In BF arrays, recordings determined to be in the SOZ showed a mean ($\pm$ SD) delay of 10.23 $\pm$ 3.03 s ($n = 6$ seizures from 5 patients), while those deemed to be in regions of spread showed a mean ($\pm$ SD) delay of 22.96 $\pm$ 12.59 s ($n = 8$ seizures, 8 patients; $p < 0.05$, Mann-Whitney U test). The 2 cases showing recruitment in dual locations showed anatomically feasible recruitment times (right hippocampal head to right hippocampal body in 1.1 s in Patient 15, and left mid-cingulate to left hippocampus in 10.3 s in Patient 14). In 1 case, single-unit waveforms remained

Figure 9. Ictal recruitment in the mesial temporal lobe recorded with BF electrodes. **Ai**, Broadband LFP from the closest macro contact to the microwires in a BF electrode in the mesial temporal lobe during a spontaneous seizure. **Aii**, The bandpass filtered MUA from one of the microwires in the temporal pole. Red dashed line indicates “global” seizure onset. Pink dashed line indicates subsequent local recruitment to the seizure. **B**, Magnification of the region denoted by the gray bar in **A**, showing seizure onset through passage of the ictal wavefront in LFP and MUA (colors maintained) in the same microwire (**Bi**), and a microwire from a nearby separate BF in the hippocampal body (**Bii**). Note the prerecruitment stability in the waveform amplitude, which is immediately reduced on ictal invasion, and the simultaneous quiescence in the hippocampal body, followed by a similar, time-delayed amplitude change after recruitment. **C**, Mean $\pm$ SD of waveforms before ictal invasion (blue) and after recruitment during the seizure (red), showing reduction in amplitude and increase in FWHM in **Aii**. **D**, Waveform amplitude versus FWHM for the defined unit, with color maintained from **A**, transitioning from blue to red through seizure invasion. Note the bimodal clusters that correspond to prerecruitment and postrecruitment, and the similarity waveform changes to those seen in Figure 5B.

Movie 1. Location-specific waveform changes during a spontaneous human seizure. Local field potential (top) and amplitudes for three units in three different locations (middle; red, blue, and purple), with associated waveforms shown to the right. Shading represents the mean $\pm$ 2 SD for these units’ preictal waveform amplitudes. The locations of the BF microwires that recorded these units are shown below, with colors maintained. Three activity patterns are as follows: cessation of firing at seizure onset in the anterior temporal lobe (blue), stability followed by loss of waveform amplitude in the anterior cingulate (red), and stability throughout in the mid-cingulate (purple) [View online]

Movie 2. Single-unit waveform alterations in an ictal BF recording. Single units undergo wave shape changes upon recruitment to the seizure, shown in real-time. Top, MUA bandpassed signal (300 Hz to 5 kHz; white). Red represents current time. Dashed magenta line indicates earliest electrographic ictal activity in the patient. Blue dashed line indicates local recruitment. Bottom, Shaded regions represent mean $\pm$ 2 SD for preictal single units in red and blue, and lower amplitude MUA in yellow (left). Waveforms are displayed in real-time, with colors matching their assigned unit, and color saturation showing the probability of a true match to that unit. The first two principal component scores for these waveforms are shown on the right, with colors maintained. Note the stability of waveforms prior to local recruitment, including after seizure onset, followed by marked loss of amplitude at the moment of recruitment with associated tonic firing. These data show another example of quiescence in one neuron, as the blue unit stops firing after seizure onset, which cannot be attributed to dropping below threshold as the background MUA (yellow) continues. [View online]

stable throughout a focal to bilateral tonic-clonic seizure (right mesial cingulate, Patient 18; Table 2).

In UMA recordings with clearly defined ictal wavefront propagation (four seizures, 2 patients) (Smith et al., 2016), the

Figure 10. Wave shape changes predict the oncoming ictal wavefront. **A**, Example spatial fits of the first sustained FWHM increase across the UMA (magenta-cyan; blue represents raw data) and the ictal wavefront as calculated by tonic firing (black-red-yellow; orange represents raw data). Note the correlation of the spatial fit across the 2D array between the two values, with FWHM increases occurring moments before the tonic firing of recruitment. **B**, Time of first sustained FWHM increase in each single unit across the population relative to the arrival of the ictal wavefront (red dashed line) at that neuron's recording channel ($n = 330$ units, 4 seizures, 2 patients). Gray triangle and bar represent mean $\pm$ SD of all units. **C**, Propagation speeds of FWHM increases spreading across the UMA, calculated from the linear regression of delays versus distance from the first electrode to show significant waveform alterations for each patient.

timings of FWHM increases correlated with the ictal wavefront timing across the arrays, with significantly higher correlation than values derived from a bootstrapping approach, shuffling the FWHM timing locations randomly 10,000 times (mean $\pm$ SD 2D correlation, observed data: $r = 0.76 \pm 0.12$; shuffled data: $r = -0.0007 \pm 0.0012$; Fig. 10). The FWHM increases preceded the tonic firing associated with ictal recruitment by an average of 1.85 s (± 1.94 s SD), and the propagation speed of FWHM changes across the arrays (mean $\pm$ SD: 0.64 mm/s $\pm$ 0.24 mm/s) corresponded with the speeds calculated previously for the ictal wavefront propagation in these patients (Schevon et al., 2012; Smith et al., 2016).

Discussion

In animal models, it is possible to isolate neurons experimentally, by guiding electrodes onto cells (Trevelyan et al., 2006). This is not possible in human recordings; and consequently, there is a dearth of rigorous evidence about the firing patterns of human neurons through seizures. Instead, previous studies have identified the ictal wavefront in terms of MUA (Schevon et al., 2012; Smith et al., 2016), used standard spike-sorting methods despite the limitations we have described (Bower and Buckmaster, 2008; Truccolo et al., 2011; Bower et al., 2012), or limited assessment to the time before seizure invasion (Misra et al., 2018).

Here, we sought to overcome these limitations, exploring the impact of action potential alterations in spontaneously occurring human seizures, how these alterations relate to underlying ictal territories, and the neuronal basis of ictal phenomena previously described in terms of MUA, such as the ictal wavefront. We previously showed that traditional spike sorting fails on ictal invasion of the recording site (Merricks et al., 2015); however, it was not possible to differentiate whether this was because of intrinsic waveform alterations or hypersynchronous activity (Trevelyan et al., 2006, 2007; Schevon et al., 2012; Weiss et al., 2013). Here, we developed novel methods to track units through seizures,

retaining identities of putative individual neurons. We hypothesized that temporary loss of clusters was due in part to transient alterations to action potential shapes, as opposed solely to obfuscation of stable waveforms by the sudden increase in MUA, and that neurons not demonstrating evidence of ictal recruitment would remain stable. The method proved remarkably effective, enabling us to define probabilistic firing rates for the majority of units throughout the seizure. This enabled us to establish that neurons only rarely become quiescent during seizures.

Units during the seizure displayed two types of activity: deformation of wave shapes across the population or largely stable waveforms. UMA recordings afforded the ability to detect local ictal recruitment through characteristic MUA firing, and these types of activity corresponded to recruitment and penumbra, respectively (Fig. 7), with all neurons that were untracked because of low ictal firing occurring in penumbra. Waveforms recovered after seizure termination and showed stereotyped responses across seizures, highlighting that a neuron's wave shape change, in response to the synaptic barrage of ictal activity, is maintained across multiple seizures.

The template-matching method would equally favor waveform alterations in any dimension, so if changes were a result of the methodology, we would expect the template to capture waveforms with larger amplitude and decreased FWHM at an equal rate. In such a case, we would anticipate a broadening of the distribution of these features during the ictal period. Instead, we see a clear shift in the distributions to the right and left in the FWHM and amplitude, respectively, arguing for a consistent physiological cause.

Detection of the ictal wavefront is generally not possible in BF arrays because of lower neuronal density in mesial structures relative to layer 4/5 neocortex (Pakkenberg and Gundersen, 1997; Keller et al., 2018), and reduced "listening spheres" because of higher impedance (BF: 50–500 k Ω ; UMA: 80–150 k Ω) (Tóth et al., 2016). Therefore, we used these recordings to evaluate the robustness of these findings, allowing for sampling of multiple

sites in a given patient. Both the presence and timing of waveform alterations correlated well with clinical observations (Table 2). Seizure spread locations showed delayed waveform changes compared with recordings from SOZ regions (22.96 vs 10.23 s), and the longest delay occurred after spread to the contralateral hippocampus (Patient 10; 42.7 s). The only case with discordance between waveform and clinical data showed an increase in FWHM during the seizure, but at no point did the mean FWHM surpass the threshold of 1 SD above the preictal mean (Patient 21). In this instance, the SOZ was in the right insula and somatosensory cortex, with waveform alterations in the right hippocampus. While hippocampal recruitment is plausible, this may represent a false positive in the temporally coarse statistical test.

In a subset of recordings, stable waveforms were found simultaneously with waveform changes both in separate BF arrays (5 seizures) and on the same UMA (1 seizure), clinical correlation matched these observations in all cases. Furthermore, in two seizures, recruitment was found at multiple BF arrays, consistent with clinical observations, along with relative delays in keeping with anatomic distance (Fig. 9; Patients 14 and 15). In the UMA, clinical observations were consistent with the array located at the outer boundary of seizure spread; we posit this is a simultaneous recording of both recruited and penumbral cortex (Fig. 8).

Also, in Patient 6, there is an increase in firing rate of waveforms of brief duration during the ictal period (Fig. 7B). Given the UMA's proximity to the oncoming ictal wavefront, this increase may be explained by an increase in firing of fast-spiking interneurons, which have been shown to exhibit brief waveforms (McCormick and Feese, 1990; Csicsvari et al., 1999; Peyrache et al., 2012) and would corroborate the penumbral feedforward inhibition model (Trevelyan et al., 2007; Cammarota et al., 2013; Parrish et al., 2019).

The waveform changes found in recruited tissue are in keeping with those observed in animal models, being indicative of the shortening and broadening of action potentials associated with PDS (Traub and Wong, 1982). This was especially evident in a unique BF array recording wherein a neuron was able to be tracked without need for extra methods because of its amplitude being 14 times that of the background noise (Patient 15; Fig. 9), with feature alterations strikingly reminiscent of the UMA population data (Figs. 5B, 9D), and timing in keeping with recruitment during tonic firing (Figs. 8 and 9).

Even so, the extent to which waveforms alter is likely underrepresented in population data because of changing detection sensitivity from interference between synchronous action potentials or reduction of amplitude of some waveforms below the noise threshold. The template-matching method was designed to minimize false negatives to capture as much single-unit activity during seizures as possible, but it is likely that waveforms undergo large enough changes to be lost outside the convex hull, or below threshold; even physiological bursting has been shown to result in substantial alterations to extracellularly recorded action potentials (Harris et al., 2000; Henze et al., 2000). As such, these results are necessarily a snapshot of the total activity of any individual neuron during the seizure, and yet still show significant waveform changes.

Together, the UMA and BF array recordings provide evidence supporting the dual-territory model of seizures: a "core" with waveform changes, coexisting with "penumbral" tissue with stable waveforms. These indicate that waveform change can be considered a defining feature of recruitment to the seizure, and the definition of recruitment is maintained at the level of single neurons.

Finally, our data demonstrate that seizures propagating through cortex are marked by tonic, local neuronal firing (Fig. 4), as opposed to the wavefront being composed of subthreshold activity because of, for example, increased K^+ concentration limiting concomitant firing. Studies of neuronal activity recorded in humans have rarely reported such a finding. Our findings in this paper suggest that extreme, rapid waveform alterations may obscure the presence of the wavefront when standard spike sorting methods are used. Despite the greatly increased sensitivity for units provided by our method, 14.6% of neurons were lost at the ictal transition, whether these ceased firing or underwent waveform changes too extreme to remain in the convex hull cannot be determined.

The method of tracking single units across the ictal transition was designed to minimize false negatives to track waveform deformations; and although firing rates were calculated in a probabilistic manner to minimize the impact of false positives, related template-matching methods, such as a Gaussian-mixture model of principal component scores, may prove useful in analyses of firing patterns. Going further, modeling of ionic currents in pathologic conditions may allow anticipation of how the waveform of each neuron should change during seizures, permitting "personalized" tracking of neurons via cell-intrinsic features that have proven useful in nonpathologic time points (Trainito et al., 2019; Mosher et al., 2020) but are disrupted during seizures.

These methods enable the use of both human and animal recordings to address open questions regarding the mechanism of seizure spread. An immediate application, given the activity presented here, is how cell types relate to the propagation of pathologic activity; considerable debate has focused on the role of interneurons in seizure spread (Grasse et al., 2013; Elahian et al., 2018; Magloire et al., 2018; Miri et al., 2018; Weiss et al., 2019). Several of these prior studies addressing cell type-specific ictal activity derived from extracellular recordings have not reported waveform alterations, suggesting that these were either recordings from penumbral territories or firing may have been substantially underestimated. We anticipate that elucidation of these mechanisms will come from data confirmed to be in recruited tissue; these methods lay important groundwork for analyses into how ictal propagation relates to the underlying firing of local inhibitory and excitatory cells.

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