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Spatiotemporal Neuronal Correlates of Seizure Generation in Focal Epilepsy

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Summary

Purpose—Focal seizures are thought to reflect simultaneous activation of a large population of neurons within a discrete region of pathological brain. Resective surgery targeting this focus is an effective treatment in carefully selected patients, but not all. While *in vivo* recordings of single-neuron (i.e., “unit”) activity in patients with epilepsy have a long history, no studies have examined long-term firing rates leading into seizures and the spatial relationship of unit activity with respect to the seizure onset zone.

Methods—Microelectrode arrays recorded action potentials from neurons in mesial temporal structures (often including contralateral mesial temporal structures) in seven patients with mesial temporal lobe epilepsy.

Key Findings—Only 7.6% of microelectrode recordings showed increased firing rates prior to seizure onset and only 32.4% of microelectrodes showed any seizure-related activity changes. Surprisingly, firing rates on the majority of microelectrodes (67.6%) did not change throughout the seizure, including some microelectrodes located within the seizure onset zone. Furthermore, changes in firing rate prior to and at seizure onset were observed on microelectrodes located outside the seizure onset zone and even in contralateral mesial temporal lobe. These early changes varied from seizure to seizure, demonstrating the heterogeneity of ensemble activity underlying the generation of focal seizures. Increased neuronal synchrony was primarily observed only following seizure onset.

Significance—These results suggest that cellular correlates of seizure initiation and sustained ictal discharge in mesial temporal lobe epilepsy involve a small subset of the neurons within and outside the seizure onset zone. These results further suggest that the “epileptic ensemble or network” responsible for seizure generation are more complex and heterogeneous than previously thought and that future studies may find mechanistic insights and therapeutic treatments outside the clinical seizure onset zone.

Keywords

multi-unit; ictogenesis; ensemble; microelectrode; electrophysiology; mesial temporal lobe

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Introduction

The guiding assumption of seizure genesis for the past fifty years has been that seizures initiate without warning when most or all neurons in an epileptic focus begin to fire synchronously (Penfield & Jasper, 1954; Kandel et al., 1991). Data collected over several decades have noted seizure-related activation of neuronal spiking both inside and outside the epileptic focus determined by the macroscopic local field potential seizure discharge (Rayport & Waller, 1967; Verzeano et al., 1971; Wyler et al., 1982; Colder et al., 1996), yet the cellular assemblies comprising the seizure onset zone (SOZ) and the cellular dynamics underlying seizure generation remain poorly understood. In part, this may be due to the limited nature of the available data: it remains a technical challenge to obtain high fidelity microwire recordings over the time span of days required to capture spontaneous seizures (Brinkmann et al., 2009), and intra-operative recordings typically capture few spontaneous seizures and are confounded by the effects of anesthesia.

Over the past decade, the acquisition, storage, and analysis of long duration, wide-bandwidth recordings from microelectrode arrays have become possible (Buzsaki, 2004; Brinkmann et al., 2009; Bower et al., 2009), and have led to the discovery of transient, pathological, local field potential (LFP) oscillations in human epileptic brain, including high frequency oscillations (HFOs) (Bragin et al., 1999; Worrell et al., 2008; Schevon et al., 2009) and microseizures (Schevon et al., 2008; Stead et al., 2010), suggesting that wide bandwidth recordings may contain additional, clinically useful information (Engel et al., 2009).

In addition to LFPs, it is now possible to obtain long term, continuous, recordings from populations of neurons and to explore the cellular correlates of spontaneous seizure generation over long time-scales and across large regions of brain tissue (Buzsaki 2004; Brinkmann et al., 2009;). When recording at the spatial scale of clinical macroelectrodes (1 – 10 mm²) the characteristics of ictal LFP discharges are often highly reproducible from seizure to seizure. The spatially reproducible nature of focal seizures in many patients with medically-resistant partial epilepsy is the basis for successful epilepsy surgery (Engel, 1996). However, unlike the stereotypical spatial patterns, the temporal dynamics of the interictal to ictal transition are more complicated. When recording from tissue volumes sampled by clinical macroelectrodes, ictal LFPs appear to begin suddenly and without warning. It is generally assumed that the neuronal correlate of seizure onset is a paroxysmal, widespread, synchronous discharge of a large population of neurons, but there is little direct evidence to support that seizures begin this way on a cellular level.

The first analyses of single unit activity during spontaneous seizures in humans with focal epilepsy found heterogeneous neuronal firing patterns at the onset of spontaneous seizures (Babb & Crandall, 1976; Babb et al., 1987). While finding a heterogeneous range of activity patterns as predicted by the “epileptic neuron” hypothesis (Wyler et al. 1982), they did not find fixed, pacemaker, or “epileptic” neurons that initiated each recorded seizure in a patient. These studies (while ahead of their time) were hampered by the technical capabilities available at that time, and focused on cellular activity at seizure onset and propagation. They were not able to rigorously address the cellular and network ensemble dynamics on longer timescales, e.g. minutes to hours, leading into spontaneous seizure. Remarkably they nonetheless found that relatively few neurons within the SOZ were actually involved in the seizure discharge, only 7% at seizure onset and no more than 40% of neurons recorded became activated even as the seizure evolved. Recently, Truccolo (Truccolo et al., 2011) investigated changes in cellular activity during the interictal-to-ictal transition by recording from a small (4×4 mm²), localized region of human epileptic neocortex using a penetrating microelectrode array (Donoghue, 2002). Similar to previous

studies, they found a heterogeneous and sparse activation of neurons during seizures (Babb & Crandall, 1976; Babb et al., 1987) and found that neurons outside the SOZ were also activated during seizures (Wyler et al., 1982). Unlike previous studies, however, they found that recurrent seizures from individual patients were stereotypical at the level of cellular recordings, with characteristic “motifs” of neuronal activations leading up to and persisting through seizures. Determining whether such neuronal firing motifs occur in mesial temporal lobe seizures and reflect the initiation of seizures requires long-term (multi-hour) recordings over a broad anatomical reach.

Enlarging the spatio-temporal scale of recordings can also help address the consistent, but still surprising result shared by all studies of human neural recordings in epilepsy: that the majority of neurons do not alter their firing rates prior to, or even in response to ongoing epileptiform LFP activity in the SOZ or surrounding tissue. The observation of sparse neuronal responses before and during interictal and ictal epileptiform discharges could reflect a distributed, “epileptic network” (Bragin et al. 2000), but it could also reflect limited spatio-temporal sampling that simply misses seizure-related activation of a fixed population of “epileptic neurons”. Differentiating between these hypotheses could guide not only studies of the mechanisms of seizure generation, but also strategies for seizure prediction and therapeutic intervention.

Materials and Methods

Data Acquisition

Patients undergoing chronic intracranial EEG (iEEG) monitoring for drug-resistant, Mesial Temporal Lobe Epilepsy (MTLE) were enrolled after informed consent was obtained in this Mayo Clinic Internal Review Board approved research protocol. Depth electrodes were implanted stereotactically into hippocampal structures as described previously (Worrell et al., 2008). Briefly, hybrid depth Pt-Ir electrodes (1.3 mm diameter, polyurethane, AD-Tech Medical Instrument Corporation, Racine, WI) containing both clinical macroelectrodes (4 or 8 contacts, 2.3 mm diameter, 5 or 10 mm spacing, 200–500 Ω) and research microelectrodes (9 or 18 oriented radially on the shaft between macro contacts and a bundle of 9 extending from the tip, 40 μ m diameter, 500–1000 k Ω) were stereotactically placed into the medial temporal lobe using an occipital or lateral approach (Van Gompel et al., 2010). High-resolution (1.5 or 3 Tesla) T₁ weighted, volumetric MRI brain scans obtained prior to implantation surgery were combined with high-resolution, 64-slice CT head scans obtained following implantation surgery, to localize macroelectrodes within the hippocampus. The location of microwire electrodes was inferred from their known relationship to clinical macroelectrodes. Local field potential (LFP) dynamics of focal seizure generation were obtained via continuous, wide-band (near DC – 9 kHz, sampling at 32 kHz, 1 μ V resolution) electrophysiological recordings using hybrid depth electrode arrays composed of clinical macroelectrodes and research microelectrode arrays. Data were acquired on a Digital Lynx system (Neuralynx Inc., Bozeman, MT), transmitted on a private ethernet line and stored for offline analysis (Bower et al., 2009; Brinkmann et al., 2009).

Determination of Seizure Onset

The seizure onset time and Seizure Onset Zone (SOZ) were determined using standard, clinical practice of expert visual review of macroelectrode recordings (MS & GAW). Candidate seizure events on continuous intracranial EEG (iEEG) recordings from both clinical macro- and research micro-electrodes were identified with an automated detector using signal line-length thresholded for hypersensitive detection, combined with clinical notes, patient event reporting, and ultimately confirmed by visual review of the iEEG recordings. Candidate events were subjected to post-hoc, expert visual review to verify or

reject detected events, while blinded to all channel and clinical information (Wilson et al., 2003; Gardner et al., 2007). The visual verification of a seizure discharge was based on the electrographical features of seizures (Schiller et al., 1998; Worrell et al., 2004; Bragin et al., 2007): 1.) Paroxysmal change in the background iEEG activity with a characteristic spectral seizure onset pattern 2.) Temporal and spectral evolution of the seizure discharge 3.) Termination of seizure discharge. The clinical seizure onset zone (SOZ) was defined by the clinical macroelectrodes that showed the earliest iEEG change at electrographic seizure onset.

After seizure onset was determined based on macroelectrode recordings, microelectrodes were grouped according to their position relative to the SOZ. Microelectrodes located between macroelectrodes in the SOZ were labeled “SOZ”, those on the same side of the brain, but outside the SOZ were labeled “Ipsilateral”, and microelectrodes in the mesial temporal lobe contralateral to the SOZ were labeled “Contralateral”.

Detection of Action Potentials

The quality of recordings was established first by visual review to identify “noisy” electrodes, which were dropped from further analysis. Data were extracted from each microelectrode and were analyzed offline to detect single neuron extracellular action potentials (i.e., to detect “spikes”). Continuous recordings (Figure 1.A) were filtered offline between 600–10,000 Hz using zero-phase-shift digital filtering. Action potentials were detected by applying a series of both absolute and relative thresholds in both voltage and time (Figure 1.B), as is commonly done in the analysis of extracellularly recorded unit activity (Harris et al. 2000). We have previously used this approach to detect action potentials during the interictal-to-ictal transition in a rodent model of epilepsy (Bower & Buckmaster 2008). In the following description, negative voltage is “down”, so that the “peak” of an action potential is in the direction of negative voltage (i.e., graphically “down”) and the “valley” is in the positive direction (i.e., graphically “up”). RMS variation (“sigma”) was computed for one minute windows and candidate action potentials identified as peak magnitudes greater than three sigma below the mean. From the most negative peak, candidate spikes increased from at least one sigma more positive than the peak value within 120 μ sec, increased at least 1.5 sigma from the peak within 150 μ sec, and reached a plateau (“valley”) within 500 μ sec. Finally, the peak-valley magnitude had to be greater than 20 μ V. The timestamps and waveforms of candidate spikes that satisfied these criteria were then stored to a MySQL database. Because these action potentials were not subsequently attributed to individual neurons, these action potentials were assumed to arise from multiple neurons (multi-unit activity, or MUA, Figure 1.C).

Binning, Peri-Event Time Histograms, and Synchrony

Seizures selected for analysis were preceded by at least two hours without seizures to avoid post-ictal changes that might confound the results. Action Potentials were binned in one minute, 30 second and 10 second bins centered on seizure onset to generate Peri-Event Time Histograms (PETHs) extending from 45 minutes prior to 15 minutes following seizure onset. Bin size did not affect results (see Table 2), so one-minute bins were chosen for all subsequent analyses. To control for differences in firing rate, firing rates were normalized to the period 45 to 30 minutes prior to seizure onset, which was called the “baseline” period (Figure 1.D). Significant changes in firing rate were detected by repeated-measures ANOVA with Dunn-Sidak correction (Sokal & Rohlf, 1995), comparing bins preceding seizure onset to firing rates observed during the baseline period.

Synchrony between action potentials on different electrodes was computed using cross-correlations (de la Rocha et al., 2007) for 10 msec bins over 1 sec and averaged for one

minute, 30 second and 10 second bins centered on seizure onset. Cross-correlations involving multi-unit activity are known to give larger values than correlations involving independent sources (Aertsen et al., 1989), so only relative comparisons were considered to be reliable. As with firing rates, significance was computed using repeated-measures ANOVA with Dunn-Sidak correction.

Results

Twelve spontaneous seizures were recorded from seven patients (N=2,2,1,2,1,2,2) undergoing intracranial monitoring for epilepsy surgery for medically refractory seizures arising from the mesial temporal lobe whose data contained multiple, low-noise microelectrode recordings. Only data from seizures separated by at least two hours of seizure-free activity were analyzed (Table 1). Of these, five patients had electrodes implanted bilaterally to determine the hemisphere in which seizures initiated. Four of the seven patients had resective surgery based on these recordings. The remaining three patients had seizures originating in both hippocampi and were not candidates for temporal lobectomy. In all seven patients, the combination of strip and depth electrodes clearly demarcated a hippocampal origin for each seizure. Recordings were obtained from a total of 207 microelectrodes (N=33, 33, 27, 33, 23, 62, 63) located on depth electrodes implanted in the mesial temporal lobe.

43 microelectrodes were located in the Seizure Onset Zone (SOZ), 85 microelectrodes were located in the same mesial temporal lobe, but not within the SOZ, and 79 microelectrodes were located contralateral to the SOZ. Wide bandwidth recordings from microelectrodes interposed between clinical macroelectrodes (Worrell et al., 2008; Stead et al., 2010) allowed the detection of action potentials from multiple, individual neurons. Spike waveforms and firing rate characteristics were computed and used to identify microelectrodes that contained MUA. Recording sessions of five patients contained two seizures and the microelectrode recordings during each seizure (here defined as a “trace”) were examined independently, producing a total of 436 ictal “traces” available for analysis. Visual review (GAW) identified 38 traces that contained too much noise and were excluded from further analysis. 136 traces had an average detection rate of less than 0.01 Hz and were dropped from future analysis, because neuron activity was too low, either because these electrodes were located in white matter tracts or because of local tissue damage. Traces with sufficiently high firing rates to allow analysis (N=262) remained stable, both in terms of firing rate and waveform shape (Table 2). 82 traces came from microelectrodes located in the SOZ, 71 traces came from the ipsilateral mesial temporal lobe, but outside the SOZ and 109 traces came from microelectrodes located in the mesial temporal lobe contralateral to the SOZ.

Only 32.4% of all traces showed seizure-related changes in action potential detection rate prior to or following the seizure onset, demonstrating that the majority of neurons were unaffected by ongoing seizures recorded from surrounding tissue. Four classes of seizure-related changes in neuron firing rate were observed (Figure 2): 1) those that showed increased firing rates prior to seizure onset (“preictal increase”, N=20, 7.6%), those with decreased detection rates prior to seizure onset (“preictal decrease”, N=25, 9.5%), 3) those with increased detection rates immediately following seizure onset (“ictal”, N=40, 15.3%), and 4) those with a constant firing rate prior to and following the seizure (“unchanged”, N=177, 67.6%). 160 traces came from microelectrodes located on the shaft of the depth electrode and 102 traces came from microelectrodes extending from the tip of the bundle, but seizure-related changes in activity were observed as frequently on microelectrodes located on the shaft (N=56 of 160, 35.0%) of the depth electrode as on microelectrodes extending from the tip (N=29 of 102, 28.4%) of the depth electrode (chi-square 2×2

contingency, $p=0.27$). Considering the locations of microelectrodes and the relative percentage of electrodes in each classification group (Figure 3), more “preictal increase” and “ictal increase” microelectrodes were observed in the SOZ and more “preictal decrease” microelectrodes were observed in the ipsilateral mesial temporal lobe than would be expected by chance ($p<0.001$, chi-square). Surprisingly, seizure-related activity of all three types was observed not just within and outside the SOZ, but also in the hippocampus contralateral to where seizures began. Mapping changes in MUA during the interictal-ictal transition to the electrode locations during a single seizure in one patient (Figure 4), seizure-related changes in activity appear to be distributed along the length of the depth electrode with some microelectrodes in the SOZ not responding to the seizure, while the largest change relative to interictal activity was observed in one of the more distal microelectrodes.

If the pathological network underlying seizure generation was a fixed neuronal assembly, then seizure-related MUA changes for the constituent neurons would be similar across seizures within a patient. Two seizures were recorded within the same, continuous recording of five patients, allowing an analysis of the inter-seizure variability of MUA. There were a total of 118 traces where seizure-related classification (e.g., “preictal increase”, see Methods) was computed for 2 different seizures. Other than showing that the “unchanged” classification was the most likely category for any given seizure, seizure-related activity was not consistent for the second seizure, regardless of categorization for the first seizure. Of 43 microelectrodes that displayed seizure-related changes in the first of two seizures, 6 showed a seizure-related change in the second (14.0%), but 3 of these microelectrodes were classified into different categories. One “preictal increase” and two “ictal increase” traces were consistent across seizures. Four microelectrodes that were classified as “unchanged” during the first seizure were classified as “preictal increase” in the subsequent seizure (i.e., “unchanged-preictal”), 15 were classified as preictal-decrease and 12 microelectrodes were classified as ictal-increase. The observation of both “unchanged” and seizure-related changes in activity from the same microelectrode during different seizures rejects the possibility that all “unchanged” microelectrodes resided in brain regions unaffected by seizures. Comparing ensemble activity between two seizures in the same patient (Figure 5), MUA patterns bear little resemblance to one another despite similar LFP recordings from a nearby macroelectrode.

Cross-correlations between groups of microelectrodes were computed for the 45 minutes preceding seizure onset to 5 minutes following onset (Figure 6). Compared to the baseline period (45–30 min prior to seizure onset), correlation coefficients differed for action potentials from microelectrodes contralateral to (0.0026 ± 0.0022), ipsilateral to (0.0044 ± 0.0023) and inside the SOZ (0.0109 ± 0.0087) during the 15 min baseline period beginning 45 min prior to seizure onset (ANOVA, $p<0.001$). Changes in synchrony were observed prior to and continuously into seizure onset only for contra-SOZ pairs, only for the last 30 sec prior to seizure onset, and not for any other groups. This suggests that a global change in action potential synchrony is not required to initiate seizures. Cross-correlations between ipsilateral and SOZ traces were increased relative to baseline following seizure onset and often persisted for several minutes, suggesting an increase in synchrony across a broad population of neurons.

Discussion

The MUA during the interictal-to-ictal transition associated with focal seizure generation showed a heterogeneous response, with MUA unchanged, increased, and decreased both inside, outside and contralateral to the SOZ. Firing rates on the majority of microelectrodes that recorded MUA, however, did not change either prior to or during the seizure. This finding was independent of anatomic location of the microelectrodes, or their relationship to

the SOZ. Even for the microelectrodes with pre-ictal MUA changes, the pattern was not repeated in subsequent seizures despite the similarity of the ictal LFP (Figures 4 & 5). Ensemble activity from two macroscopically similar seizures (Figure 5) suggest that the neuronal assemblies underlying their generation do not necessarily overlap and that neurons that might be considered part of the epileptic network during one seizure may not fire at all during a subsequent seizure. Finally, the absence of increased global synchrony (Fig. 6) suggests that any change in synchrony prior to seizure onset occurs within a restricted population of neurons.

This heterogeneous pattern of neuronal firing in seizure generation agrees with previous observations during spontaneous seizures from both humans (Babb & Crandall, 1976) and epileptic rats (Bower & Buckmaster, 2008). The firing rates of granule cells in epileptic rat Dentate Gyrus displayed heterogeneous seizure-related firing rates, and while a group of units showed increased firing at seizure onset, the largest group showed no change at all prior to or during the seizure (Bower & Buckmaster, 2008). Unlike earlier human studies of neuronal activity at and following seizure onset (Verzeano et al., 1971; Babb & Crandall, 1976), the increased spatial sampling and longer recording durations in this study provide a systematic test of whether specific, “epileptic” neurons become active prior to seizures based on neuronal firing rates during long (> 1 hour) interictal periods and compared to the interictal-ictal transition. The strict action potential detection criteria used in this study could produce instances of false negative action potentials (i.e., “missed spikes”), but it is unlikely to have affected the results, because missed action potentials would bias towards decreased firing rates, particularly around the time of seizures when the extracellular concentrations of ions may change, thus distorting waveforms. Our data contain several clear examples of increased firing rates in all patients. Therefore, if the current approach of spike detection did bias detection rates, our results should give a more conservative estimate of single neuron firing increases prior to seizures.

The observation that the majority of recorded neurons are not involved in seizures and that “preictal increase” microelectrodes are present inside and outside the SOZ (Figure 3) suggests a more spatially distributed and sparse network of neural tissue is involved in ictogenesis. The observation that more “ictal” neurons were observed in the SOZ than would be predicted by chance, however, is consistent with the SOZ as the site of initial seizure propagation. The observation of increased “preictal decrease” responses ipsilateral to, but outside of the SOZ could reflect an inhibitory surround response at seizure onset, but it should be noted that this result is heavily weighted by observations from a single patient (Patient 4, Table 2). The presence of all types of responses in all three categories of tissue (SOZ, ipsi and contra) argues against the assumption, however, that ictogenesis occurs solely in the SOZ.

Seizures are defined by the spatiotemporal characteristics of pathological local field potentials, and the emphasis on macroelectrode recordings and success of epilepsy surgery has fostered these definitions. Epilepsy, however, is a disease of cells - neurons and glia - not field potentials, and the concept that focal seizures originate from a well defined population of neurons within a fixed epileptic network is not necessarily what should be expected from neuronal microcircuitry. The results do not support a model of focal epileptic brain and ictogenesis characterized by a pathological collection of neurons localized to the SOZ that become highly synchronized and serve as fixed, seizure-generating “seeds” that recruit the majority of surrounding neurons. The findings in this study are consistent with seizures arising from an “epileptic network” with multiple neuronal assemblies within that network capable of seizure initiation from multiple locations that evolve into the stereotypic, macroscopic ictal LFP.

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References

- Aertsen AM, Gerstein G, Habib MK, Palm G. Dynamics of neuronal firing correlation: modulation of "effective connectivity". *J Neurophysiol.* 1989; 61:900–917. [PubMed: 2723733]
- Babb TL, Crandall PH. Epileptogenesis of human limbic neurons in psychomotor epileptics. *Electroencephalogr Clin Neurophysiol.* 1976; 40:225–243. [PubMed: 57852]
- Babb TL, Wilson CL, Isokawa-Akesson M. Firing patterns of human limbic neurons during stereoencephalography (SEEG) and clinical temporal lobe seizures. *Electroencephalogr Clin Neurophysiol.* 1987; 66:467–482. [PubMed: 2438112]
- Bower MR, Buckmaster PS. Changes in granule cell firing rates precede locally recorded spontaneous seizures by minutes in an animal model of temporal lobe epilepsy. *J Neurophysiol.* 2008; 99:2431–2442. [PubMed: 18322007]
- Bower MR, Stead M, Brinkmann BH, Dufendach K, Worrell GA. Metadata and annotations for multi-scale electrophysiological data. *Conf Proc IEEE Eng Med Biol Soc.* 2009; 2009:2811–2814. [PubMed: 19964266]
- Bragin A, Claeys P, Vonck K, Van Roost D, Wilson C, Boon P, Engel J. Analysis of initial slow waves (ISWs) at the seizure onset in patients with drug resistant temporal lobe epilepsy. *Epilepsia.* 2007; 48:1883–1894. [PubMed: 17559569]
- Bragin A, Engel JJ, Wilson CL, Fried I, Buzsaki G. High-frequency oscillations in human brain. *Hippocampus.* 1999; 9:137–142. [PubMed: 10226774]
- Bragin A, Wilson CL, Engel J. Chronic epileptogenesis requires development of a pathologically interconnected neuron clusters: a hypothesis. *Epilepsia.* 2000; 41:144–152.
- Brinkmann BH, Bower MR, Stengel KA, Worrell GA, Stead M. Large-scale electrophysiology: acquisition, compression, encryption, and storage of big data. *J Neurosci Methods.* 2009; 180:185–192. [PubMed: 19427545]
- Buzsaki G. Large-scale recording of neuronal ensembles. *Nat Neurosci.* 2004; 7:446–451. [PubMed: 15114356]
- Colder BW, Frysinger RC, Wilson CL, Harper RM, Engel J. Decreased neuronal burst discharge near site of seizure onset in epileptic human temporal lobes. *Epilepsia.* 1996; 37:113–121. [PubMed: 8635420]
- de la Rocha J, Doiron B, Shea-Brown E, Kresimir J, Reyes A. Correlation between neural spike trains increases with firing rate. *Nature.* 2007; 448:802–806. [PubMed: 17700699]
- Donoghue JP. Connecting cortex to machines: recent advances in brain interfaces. *Nat Neurosci.* 2002; (5 Suppl):1085–1088. [PubMed: 12403992]
- Engel J, Bragin A, Staba R, Mody I. High-frequency oscillations: what is normal and what is not? *Epilepsia.* 2009; 50:598–604. [PubMed: 19055491]
- Engel, J. Principles of epilepsy surgery. In: Shorvon, S.; Dreifuss, F.; Fish, DR., editors. *The treatment of epilepsy.* Oxford: Blackwell; 1996. p. 519-529.
- Harris KD, Henze DA, Csicsvari J, Hirase H, Buzsaki G. Accuracy of tetrode spike separation as determined by simultaneous intracellular and extracellular measurements. *J Neurophysiol.* 2000; 84:401–414. [PubMed: 10899214]
- Gardner A, Worrell GA, Marsh E, Dlugos DJ, Litt B. Human and Automated Detection of High-Frequency Oscillation in Clinical Intracranial EEG Recordings. *Clin Neurophysiol.* 2007; 118:1134–1143. [PubMed: 17382583]
- Kandel, E.; Schwartz, J.; Jessell, T. *Principles of neural science.* 3 ed. Norwalk, CT: Appleton and Lange; 1991.
- Penfield, W.; Jasper, H. *Epilepsy and the functional anatomy of the human brain.* Boston, MA: Little Brown; 1954.

- Rayport M, Waller HJ. Technique and results of micro-electrode recording in human epileptogenic foci. *Electroencephalogr Clin Neurophysiol*. 1967; (Suppl. 25):143–151. [PubMed: 4165778]
- Schevon CA, Ng SK, Cappell J, Goodman RR, McKhann G, Waziri A, Branner A, Sosunov A, Schroeder CE, Emerson RG. Microphysiology of epileptiform activity in human neocortex. *Clin Neurophysiol*. 2008; 25:321–330.
- Schevon CA, Trevelyan AJ, Schroeder CE, Goodman RR, McKhann G, Emerson RG. Spatial characterization of interictal high frequency oscillations in epileptic neocortex. *Brain*. 2009; 132:3047–3059. [PubMed: 19745024]
- Schiller Y, Cascino GD, Busacker NE, Sharbrough FW. Characterization and comparison of local onset and remote propagated electrographic seizures recorded with intracranial electrodes. *Epilepsia*. 1998; 39:380–388. [PubMed: 9578028]
- Sokal, RR.; Rohlf, FJ. *Biometry : the principles and practice of statistics in biological research*. New York: W.H. Freeman; 1995.
- Stead M, Bower MR, Brinkmann BH, Lee K, Marsh WR, Meyer FB, Litt B, Van Gompel J, Worrell GA. Microseizures and the spatiotemporal scales of human partial epilepsy. *Brain*. 2010; 133:2789–2797. [PubMed: 20685804]
- Truccolo W, Donoghue JA, Hochberg LR, Eskandar EN, Madsen JR, Anderson WS, Brown EN, Halgren E, Cash SS. Single-neuron dynamics in human focal epilepsy. *Nat Neurosci*. 2011; 14:635–641. [PubMed: 21441925]
- Van Gompel JJ, Meyer FB, Marsh WR, Lee KH, Worrell GA. Stereotactic electroencephalography with temporal grid and mesial temporal depth electrode coverage: does technique of depth electrode placement affect outcome? *J Neurosurg*. 2010; 113:32–38. [PubMed: 20170311]
- Verzeano M, Crandall PH, Dymond A. Neuronal activity of the amygdala in patients with psychomotor epilepsy. *Neuropsychologia*. 1971; 9:331–344. [PubMed: 4948323]
- Wilson SB, Scheuer ML, Plummer C, Young B, Pacia S. Seizure detection: correlation of human experts. *Clinical neurophysiol*. 2003; 114:2156–2164.
- Worrell GA, Gardner AB, Stead SM, Hu S, Goerss S, Cascino GJ, Meyer FB, Marsh R, Litt B. High-frequency oscillations in human temporal lobe: simultaneous microwire and clinical macroelectrode recordings. *Brain*. 2008; 131:928–937. [PubMed: 18263625]
- Worrell GA, Parish L, Cranstoun SD, Jonas R, Baltuch G, Litt B. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain*. 2004; 127:1496–1506. [PubMed: 15155522]
- Wyler AR, Ojemann GA, Ward AA. Neurons in human epileptic cortex: correlation between unit and EEG activity. *Ann neurol*. 1982; 11:301–308. [PubMed: 7092182]

Figure 1.

Long-duration, high-frequency recording of multi-unit activity (MUA) A) 50 min of continuous data recorded from a macro- and micro-electrodes (depth electrode schematic shown in inset, described in Worrell et al. 2008) in hippocampus beginning 45 min prior to seizure onset ("0"). The hybrid depth electrodes are composed of 4 or 8 clinical macroelectrodes (blue), 9 microwires (black) exiting the tip of the depth, and 18 microwires arranged between the clinical macroelectrodes along the depth electrode shaft. B) Action potentials were detected using a series of both relative and absolute thresholds in both voltage and time. Candidate action potentials had to satisfy each of the six criteria, or else they were rejected: a. peak > three standard deviations ("three sigma"), b. keep largest value,

c. rise to peak > 1 sigma, d. fall from peak > 1.5 sigma, e. peak-valley time between .03–.5 msec, f. $|\text{peak-valley}| > 20 \mu\text{V}$. C) Expanded view showing 5 seconds of filtered (600–10,000 Hz) microelectrode data (C1) 45 min prior to seizure onset with action potentials marked by “.” and an example shown at right (C2) seconds prior to seizure onset with a rejected action potential marked by “x”, each accepted action potential detection marked (“.”) and an example shown at right. Example action potentials are shown at right on a 5 msec timescale. D) Firing rate for the time period shown in panel A for action potentials shown in panel B. The activity on this microelectrode was classified as “preictal increase”.

Figure 2.

Seizure-related activity observed for all recorded data. Each row shows a peri-event (seizure) time histogram (PETH) with one-min bin width for one microelectrode around one seizure (i.e., a “trace”) beginning 45 min prior to seizure onset. Grayscale is normalized to firing during 15 min beginning 45 min prior to onset ranging from black (no firing) to white (twice the baseline firing rate or greater). Traces from the SOZ are at top, ipsilateral MTL outside SOZ middle, and contralateral MTL bottom. Rows are grouped by seizure-related firing rate changes: hollow arrows for preictal changes, solid arrows for post-ictal changes and “NC” for “no change”.

Figure 3.

All types of seizure-related firing rate changes were observed in all recorded brain regions. For each activity-related group, grey bars show the expected relative ratios by each anatomical grouping (i.e., grey bars in each group sum to 100%). Colored bars placed on top show the actual percentage of contralateral (blue), ipsilateral outside SOZ (green) and SOZ (red) relative ratios. While SOZ microelectrodes predominate in the preictal-increase and ictal-increase groups and ipsilateral microelectrodes dominate the preictal-decrease group, examples of each seizure-related activity change are observed in all anatomical groups.

Figure 4.

Seizure-related activity on microelectrodes is highly variable with regards to Seizure Onset Zone. (LEFT) Schematic of a depth electrode for one seizure containing macroelectrodes (bars), microwires extending from the tip of the electrode (lines) and microwires placed between macroelectrodes on the shaft (dots). Light gray rectangle shows the clinically defined Seizure Onset Zone (SOZ) as determined by low-frequency clinical criteria. Arrows from macroelectrodes and circled microelectrodes point to EEG shown on the right. (RIGHT) EEG and firing rates beginning 45 min prior to seizure onset ("0"). EEG for microelectrodes has been high-pass filtered to show unit activity, but seizures were clearly observed on microelectrodes near the SOZ commensurate with seizure activity observed on

the macroelectrodes. Vertical scale is the firing rate normalized to firing rate during 15 min beginning 45 min prior to seizure onset. Note that the scale on the top row is much larger than the other rows. Symbols at right show seizure-related activity change category: hollow arrows show pre-onset changes, solid arrows show post-onset changes and “NC” shows “no change”.

Figure 5.

Seizure-to-seizure variability, despite similar macroscopic recordings. Top and bottom panels show 45 sec of data around seizure onset from sequential seizures during the same recording session, separated by 5 hours. EEG recording on the top line of each panel shows data from the same macroelectrode during each seizure. Colored plots below show firing rates for 30 microelectrodes, normalized to the firing rate of each electrode for 15 min beginning 45 min prior to seizure onset. Flat lines show microelectrodes that recorded firing during the baseline period, but showed little or no activity during the seizure.

Figure 6.

No change in cross-correlation observed prior to seizure onset. Traces were grouped according to the location of the microelectrode relative to the macroelectrode-determined seizure onset zone: contralateral to (Contra), ipsilateral to (Ipsi) and within (SOZ) the seizure onset zone. (A) Average time-shift normalized cross-correlation across all patients. Significant correlations observed prior to seizure onset did not persist continuously up to seizure onset. (B) Example from one patient showing no pre- or post-ictal changes in cross-correlation.

Table 1

Detailed Patient Information

Pt#	Intracranial Electrodes	Interictal EEG Location	Seizures (Anatomic location of IOZ)	MRI	Surgery, Pathology and Outcome
1 Age Onset: 12 y Age: 58 y RF: Febrile Seizures	Right and Left Temporal Depths: RTD & LTD 8-contact hybrid	Bitemporal: LTD 1,2,3,4 RTD 1,2	L. Temporal Seizures LTD 4,5 (HC) R. Temporal Seizures RTD 1,2 (AMD & HC)	Bilateral HC Atrophy L>R	Resective Surgery: No Path: N/A Outcome: N/A
2 Age Onset: 13 y Age: 37 y RF: None	Left Temporal Strips: 3 Neocortical 8 contact strips (superior, middle and inferior temporal gyrus) Depths: AD & PD hybrids	Left Temporal: AD 1,2,3,4 PD 1,2,3	#1 Left Temporal: AD 1,2 PD 1,2	Normal	Resective Surgery: L. ATL Path: Gliosis Outcome: IL/AE-1 F/u = 18 months
3 Age Onset: 3 y Age: 27 y RF: Bacterial Meningitis 3 y	Right and Left Temporal Depths: RTD & LTD 8-contact hybrid	n/a	R. Temporal Seizures RTD 1,2 (AMD) L. Temporal Seizures LTD 1,2 (HC)	Bilateral HC Atrophy (L>R)	Resective Surgery: No Path: N/A Outcome: N/A
4 Age Onset: 38 y Age: 47 y RF: None	Right and Left Temporal Depths: RTD & LTD 8-contact hybrid	Bitemporal: LTD 1,2 RTD 1,2	Left Temporal Seizures LTD 1,2 (AMD & AHC) Right temporal Seizures RTD 1,2,3,4 (AMD & AHC)	Normal	Resective Surgery: No Path: N/A Outcome: N/A
5 Age Onset: 15 y Age: 22 y RF: Febrile Convulsion 9 months	Right and Left Temporal Depths: RTD & LTD 8-contact hybrid	Bitemporal: LTD 1,2,3,4 RTD 1,2	L. Temporal Seizures LTD 1,2 (AHC)	Bilateral HC Atrophy and T2 signal L>R	Resective Surgery: L. ATL Path: MTS Outcome: IL/AE-1 F/u = 18 months
6 Age Onset: 22 y Age: 58 y RF: Closed head injury with convulsion at age 13 y	Left Temporal Strips: Three 1x8 strips (superior(LSS), middle (LMS) and inferior (LIS) temporal gyrus) Depths: AD & PD 4-contact hybrids	L. Temporal: LMS 2 AD 1,2,3,4 PD 1,2,3	L. Temporal: AD 1,2 PD 1,2 LMS 2,3	Normal	Resective Surgery: L. ATL Path: Subpial gliosis Outcome: IL/AE-1 F/u = 24 months
7 Age Onset: 12 y Age: 21 y RF: None	Left Temporal Grid: 24 contact (4x6) Strip: 1x8 Anterior Temporal (ATS) Depths: AD, MD & PD 4-contact hybrids	L. Temporal: ATS 1,2,3,4 AD 1,2 MD 1,2	L. Temporal Seizures AD 1,2 (AMD) MD 1,2 (AHC)	Normal	Resective Surgery: L. Temporal Cortisectomy. Path: Subpial gliosis Outcome: IL/AE-1 F/u = 28 months

Posterior approach depth electrode: 8 macrocontacts and 18 microwires. Lateral approach depth electrode: 4 macrocontacts and 20 microwires. RTD, right temporal depth; LTD, left temporal depth; AD, anterior depth; MD, middle depth; PD, posterior depth. ATL, anterior temporal lobectomy; Amygdala (AMD), Hippocampus (HC), Entorhinal cortex (EC), Mesial Temporal Sclerosis, Ictal onset Zone (IOZ), non-ictal onset zone (nIOZ), microseizure (μ Sz), micro-periodic epileptiform discharge (μ PED), International League Against Epilepsy (ILAE) outcome score (20).

Table 2

Percentage of electrodes providing seizure-related information

Patient	1	2	3	4	5	6	7	all
Count								
total	66	66	27	66	23	62	126	436
good	63	66	26	50	19	58	116	398
rate	46	58	13	40	9	40	56	262
pre_inc	3/3/2	1/1/1	3/4/4	2/2/2	3/1/0	2/1/1	6/7/7	20/19/17 (7.6%)
pre_dec	0/0/0	2/1/0	1/0/0	14/15/12	1/1/0	0/0/0	7/8/7	25/25/19 (9.5%)
ict_inc	5/0/0	28/22/11	0/0/1	1/5/0	5/6/5	1/4/2	0/2/3	40/39/22 (15.3%)

For each patient, “total” describes the number of electrodes times the number of recorded seizures. Visual inspection identified “good” quality recordings. Following detection of action potentials, electrodes with sufficient firing “rate” were grouped into seizure-related classes: “pre_inc”/“pre_dec” recordings with significantly increased/decreased firing prior to seizure onset and “ict_inc” recordings with significantly increased firing following seizure onset. Seizure-related changes in activity were computed using three different bin sizes: one minute, 30 seconds and 10 seconds and the results for each bin size are arranged in the table as one-minute / 30 sec / 10 sec.