Evidence of an inhibitory restraint of seizure activity in humans

Supplementary Information

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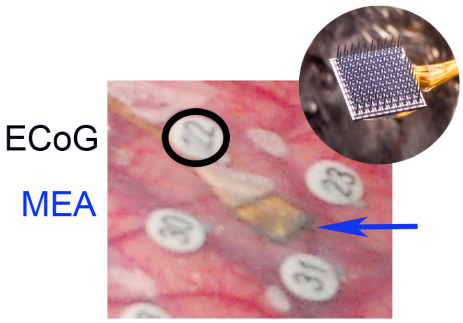
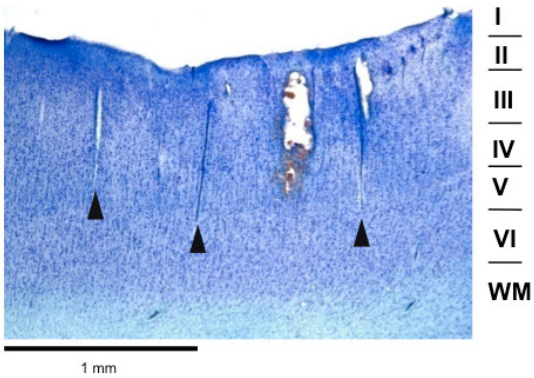
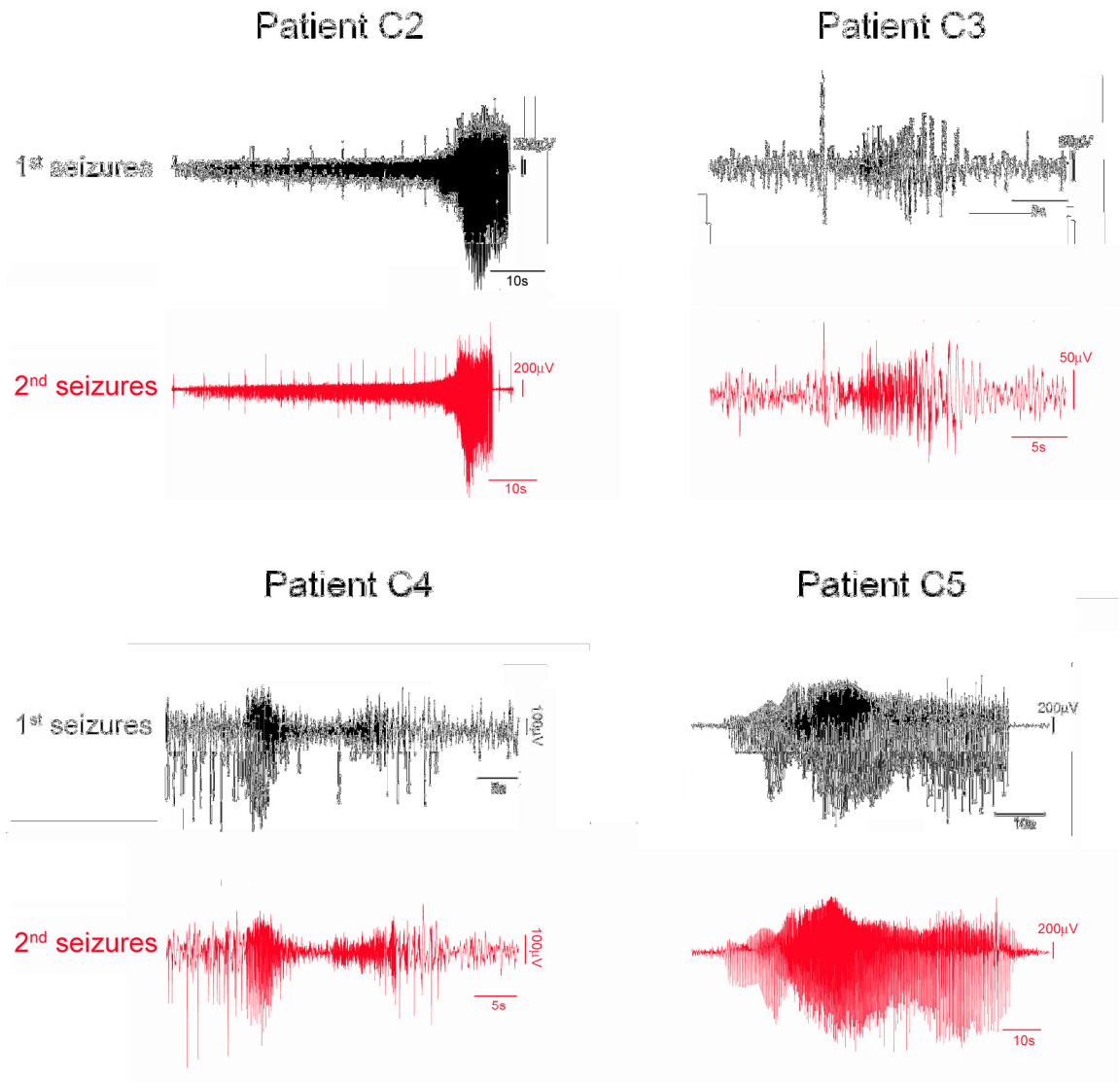


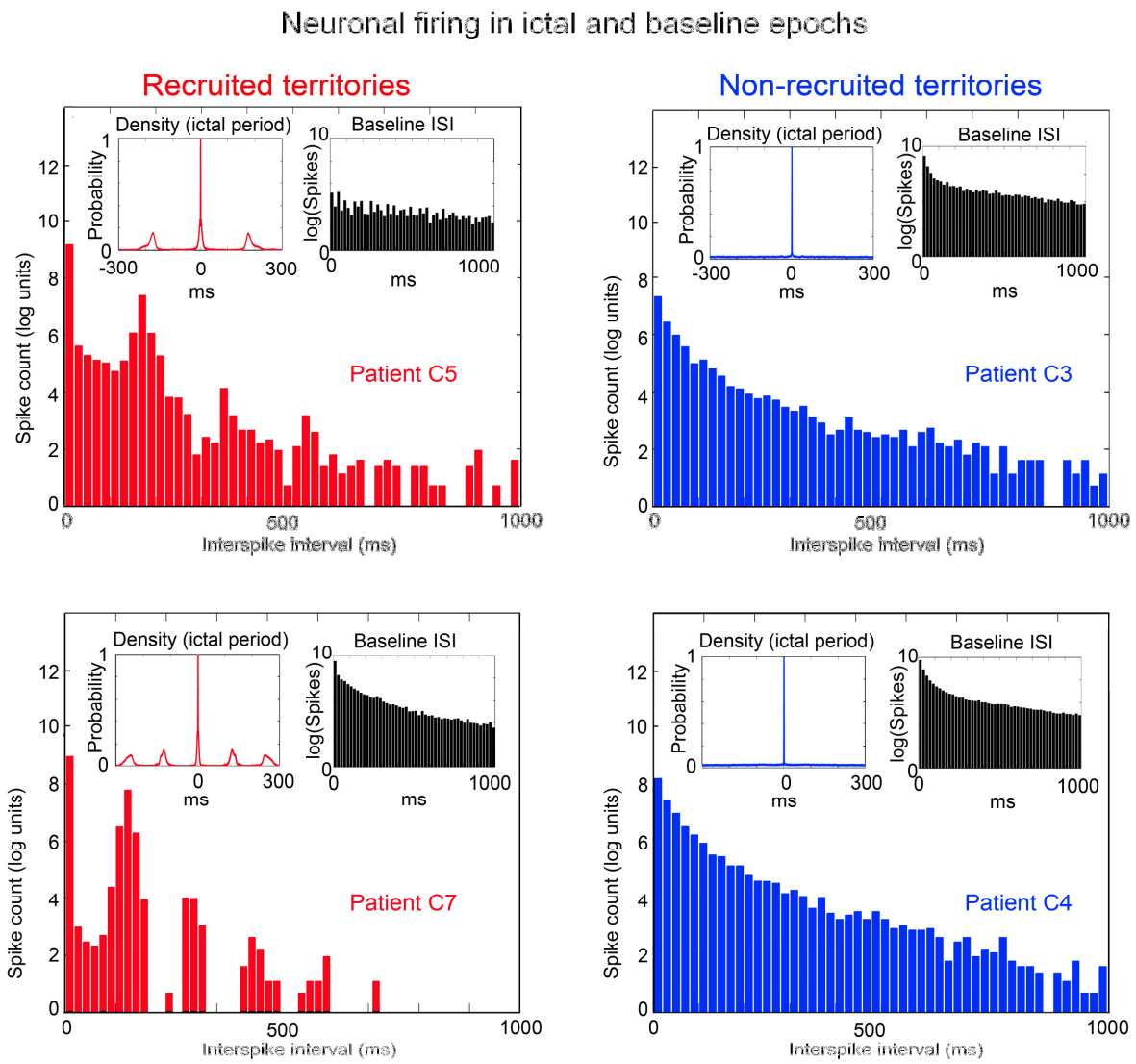
Supplementary Figure S1. Simultaneous EEG and MEA implantation. An intraoperative photo from Patient C7 showing the placement of the MEA (inset, blue arrow) underneath the subdural grid, surrounded by four electrodes all of which were within the seizure onset zone. The origin of the ictal wavefront in the MEA ("recruitment index" channel in Figure 4A) was on the side closest to subdural electrode 22 (black circle), which was among the electrodes clinically designated as seizure onset. The MEA's exiting gold wire, which marks the edge that we place on the left side by convention, can be seen passing alongside electrode 22.



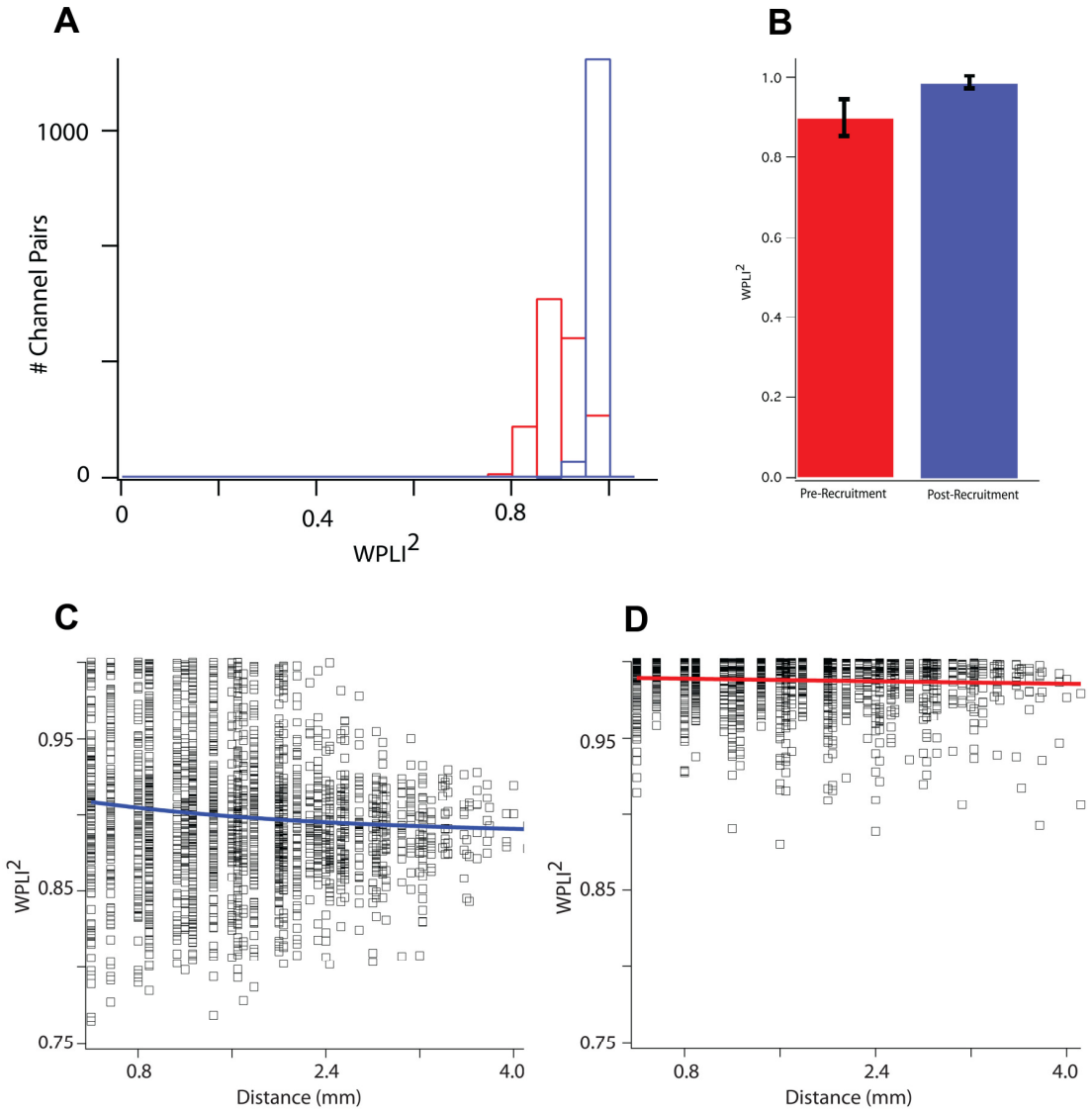
Supplementary Figure S2: Nissl-stained section from patient C4's MEA implant site. The electrode tracks are clearly visible (black arrows), and indicate the position of electrode recording tips in layers 4-5.



Supplementary Figure S3. Stereotyped low frequency (EEG) patterns in microelectrode recordings from epilepsy patients. We show traces from the same channel during two seizures for the four patients who experienced multiple episodes during the recordings. Note the variations between patients, but the strikingly reproducible electrophysiological structures in any single patient.



Supplementary Figure S4. Multiunit firing density and interspike intervals averaged across channels. Analyses from preictal baseline periods (3 min) are shown as insets in each panel (black histograms), and for ictal periods for the two groups of recordings: recruited territories (C5 and C7, for periods after the wavefront passage; red traces) and non-recruited territories (C3 and C4; blue traces). For all baseline samples, firing probability is close to zero within 300ms of each detected spike, and interspike intervals follow a classic Poisson distribution. This pattern was maintained throughout the seizure period for C3 and C4. In contrast, in the other two patients, C5 and C7, there was a marked change to rhythmic firing, consistent with the phase analyses (Figure 5 in the main paper).



Supplementary Figure S5. WPLI (nonzero phase lag coherence) during seizures. A) Histogram of WPLI values computed for all channel pairs show more variability and lower values in penumbral recordings (C3 and C4; red) vs. post-recruitment (C5 and C7; blue). B) Bar graph depicting mean (solid bars) and standard deviation (error bars) values for the two conditions. C,D) Scatterplots of pre-recruitment (C) and post-recruitment (D) WPLI values vs. interelectrode distance with exponential data fitting (solid lines) illustrating minimal distance-dependence of the WPLI measure within the region sampled by the MEA.

Patient (age/gender)	C2 (30F)	C3 (30M)	C4 (39M)	C5 (32F)	C7 (19F)
Implant Location	Right parietal including central sulcus, extending back to occipital lobe and inferiorly to posterior temporal region	Left lateral frontal, mesial frontal, temporal	Left lateral and mesial frontal	Left lateral and subtemporal	Right lateral and subtemporal, parietal, occipital
MEA Location	Right parietal 4cm posterior to central sulcus	Left supplementary motor area, 3 cm superior to Broca's area	Left lateral frontal 2 cm superior to Broca's area	Left inferior temporal gyrus 2.5 cm from anterior temporal pole	Right posterior temporal, 1 cm inferior to angular gyrus
Seizure Onset Zone	Right parietal (8x2cm cortical area, including MEA site)	Left supplementary motor area (including MEA site)	Left frontal operculum (3x3 cm cortical area, including MEA site)	Left basal/anterior temporal, including MEA site	Right posterior lateral temporal, including MEA site
Days Recorded	2	4	4	5	28
Number of Seizures Captured	39	22	7	3	1
Seizure Type(s)	Simple and complex partial	Complex partial/tonic	Complex partial	Complex partial	Complex partial with secondary generalization
Pathology	Nonspecific	N/A (multiple subpial transections performed)	Nonspecific	mild CA1 neuronal loss; lateral temporal nonspecific	Nonspecific
Outcome (> 2 years)	Engel III	Engel III	Engel 1a	Engel 1a	Engel 1a
Notes	Status epilepticus with cyclic seizures throughout recording period	Possible additional epileptic focus in left temporal lobe			

Supplementary Table S1. Clinical and demographic data for the five patients. The two patients in whom seizures were not recorded (one did not have seizures for two weeks, and the surgical decision was made based on interictal data; there were technical difficulties with the MEA recording in the other patient after the first 24 hours) are not shown. Patient C2 was in complex partial status epilepticus with cyclic (recurrent) seizures throughout the 2 days of monitoring; this was similar to frequent episodes of status epilepticus noted both clinically and on scalp recordings prior to implantation. Patient C3 underwent multiple subpial transections in the left supplementary motor area (including the MEA implantation site) due to concerns about a possible additional epileptic focus in the left temporal lobe. "Nonspecific" pathology includes effects of the subdural array implantation such as Chaslin's marginal gliosis.

	No. channels	Preictal	Early stage	Dip (minimum)	Late stage
Recruiting seizures (N=4)	165	0.83 +/- 0.17	0.93 +/- 0.09	0.87 +/- 0.05	0.98 +/- 0.004
Non-recruiting seizures (N=12)	612	0.85 +/- 0.15	0.89 +/- 0.06	0.81 +/- 0.06	0.84 +/- 0.08

Supplementary Table S2. Results of coherence calculations in 2-50 Hz MEA recordings, showing mean and standard deviation for all recording channels. Mean coherence in the early seizure stage is mildly increased from the preictal (2 min immediately preceding seizure onset) values; low standard deviation reflects the regular rhythms that characterize seizures. Coherence then decreases from the early to late seizure stages in non-recruiting events, but increases in recruiting seizures. Both findings are significant at $p < 0.001$ (paired t-test by channel with Bonferroni correction). A transient decrease, or dip, in the coherence values is present between early and late stages in both recruiting and non-recruiting seizures. Again, the dip is significant in both cases.

	Seizures analyzed	Channels	Recruited (R) / Non recruited (NR)	Multiunit correlation	Low frequency correlation
C2	5	43	R	0.84 +/- 0.09	0.76 +/- 0.16
C5	3	84	R	0.71 +/- 0.14	0.93 +/- 0.05
C3	5	11	NR	0.30 +/- 0.28	0.37 +/- 0.33
C4	7	30	NR	0.38 +/- 0.26	0.42 +/- 0.33

Supplementary Table S3. Stereotyped unit and low frequency activity distinguishes recruiting from non-recruiting seizures. Cumulative multiunit and low frequency (2-50 Hz) activity for channels averaging at least 12 spikes per second across all seizures were calculated for this table and for Figure 4 in the main text. The relatively low number of channels for C3 and C4 reflect the lower levels of spiking activity and the lack of consistent firing patterns across seizures in these patients. Mean and standard deviation of the Spearman correlation coefficients for both multiunit and low frequency activity are significantly different in recruiting (C2, C5) vs. non-recruiting (C3, C4) events (Mann-Whitney test, $p < 0.05$).

Supplementary Methods

Patient Recordings – Detailed Methods and Clinical Background

Device information: The microelectrode array (MEA) used in this study is an FDA-approved device (Neuroport™ neural monitoring system, Blackrock Microsystems, Salt Lake City, UT) that has been safely implanted in humans at several institutions[17-19]. The array measures 4 mm x 4mm, and contains 96 microelectrodes arranged in a regular 10 x 10 square with no electrodes at the corner positions (Supplementary figure 1). The individual microelectrodes were platinum-coated silicon, protruding 1 mm from the array base and were electrically insulated except for the terminal 70μm. They tapered from 35-75μm in diameter at the base to 3-5μm at their recording tips. Electrode impedance at manufacture was 322 +/- 138 kΩ. The electrode was designed to record from layers 4 and 5.

Implantation and clinical methods: The MEA was implanted alongside subdural and depth electrodes into the neocortex of patients with medically intractable focal epilepsy undergoing intracranial EEG recording at the Columbia University Medical Center/New York-Presbyterian Hospital to help identify the epileptogenic zone, i.e. the tissue that must be removed to obtain seizure control. Use of the MEA was limited to patients for whom the presurgical evaluation indicated clear seizure localization to a restricted region, in whom invasive recording was performed to refine the resection boundaries, in order to ensure that the implantation site was included in the area targeted for subsequent surgical treatment. Cases that were considered appropriate included temporal lobe epilepsy, in which the implantation was performed to define the contribution of lateral temporal neocortex and tailor/extend temporal lobectomy, and extratemporal syndromes in which scalp EEG recording indicated a consistent and well-defined interictal and ictal focus limited to a sublobar distribution and confirmed by a neuroimaging study. The study was approved by the Institutional Review Board of the Columbia University Medical Center and informed consent was obtained from each patient prior to the procedure. All 7 patients offered the opportunity to participate in this study agreed to do so. The MEA implantation site was within the seizure onset zone in 6 of 7 cases, and was subsequently treated either with corticectomy or multiple subpial transections. In the remaining case (patient C1), the seizure onset zone was deemed to be mesial temporal, and the MEA site was within the anterolateral temporal resection area.

The MEA was implanted into flat surfaces of exposed neocortical gyri through the pia mater using a pneumatic insertion technique[17]. The implant site was selected based on the estimation of the epileptogenic region from presurgical studies, as described above. Particular care was taken to place the array within territory that prior assessments had indicated should be removed as part of the surgical treatment. Lateral temporal sites were chosen to fall within the region to be included in anterolateral temporal lobectomy (ATL), the minimum resection expected to be carried out, as in these cases implantation is done to determine whether extension of the standard ATL is needed to control seizures. Extratemporal implantation sites were selected from regions with prominent interictal epileptiform discharges identified by intraoperative corticography, a standard clinical procedure during subdural electrode implantation at our institution. Potentially eloquent sites such as Broca's area or primary motor cortex were avoided. Following MEA implantation, standard clinical macroelectrode grids were placed. The MEA assembly includes two reference wires; one was placed subdurally near the MEA, the other epidurally. The reference signal was selected to optimize recording quality; most often, it was found that best results were obtained with the epidural reference. To monitor recording integrity, electrode impedance testing and visual inspection of sample data was performed after the initial hookup and daily thereafter.

Following implantation, the patients were first observed overnight in the Neurological ICU, then transferred to the Epilepsy Monitoring Unit where both MEA and clinical recordings were initiated. Antiepileptic medication was gradually withdrawn beginning post-implantation day 2 in

order to provoke habitual seizures, if necessary. The reduction schedule was tailored to each patient's individual medical regimen, and on daily review of the recorded EEG. One medication was reduced at a time, over a period of 2-3 days. Medication reduction was slowed or stopped if the EEG background contained a heavy burden of epileptic activity, or if spontaneous seizures occur. In the case of patient C2, additional medications were given in an attempt to control the status epilepticus.

Simultaneous MEA and clinical recordings were obtained during the entire monitoring period, and both were archived to Jetstore RAID drives (Hewlett-Packard, Palo Alto, CA) with dual tape backup. MEA data were de-identified at acquisition; XLTek data were postprocessed into the Persyst format, to permit random access, and simultaneously de-identified. The size of the entire dataset is 26 terabytes (uncompressed), although patient C7's data accounts for over half of this due to the extended monitoring period of 28 days.

Seizures were detected by clinical symptoms noted by patient or staff, combined with exhaustive visual EEG review carried out initially by the clinical team daily during the monitoring period, and repeated post-hoc by a neurophysiology-qualified investigator (CAS). Seizure onset times were determined based on the EEG recording, first by the clinical team, then confirmed by consensus of two neurophysiologists (CAS and RGE). The seizure onset and offset were defined using standard electrographic criteria. Onset was defined as the earliest occurrence of a pattern that immediately precedes the patient's clinical seizure, and that was not seen interictally. Offset was taken as the time at which the evolving, developed ictal rhythm ceased, which usually occurred abruptly over either the entire area affected by the seizure, or a large subset of it. Seizure onsets in MEA recordings were determined through the alignment with the EEG study. Following the monitoring period, with duration determined by clinical needs, the MEA was explanted along with the clinical grids. Cortical resection of the epileptogenic zone as determined by the clinical team was carried out at the time of explant.

Histology: Tissue samples from the MEA implant sites were examined in all patients, and more extensive testing was conducted in three patients (C4, C5 and C7) to assess both tissue condition and the position of the electrode tips. Each specimen was fixed in paraformaldehyde with the MEA in place, and slices were treated with Nissl, GFAP and Vimentin stains. As all cortical samples were taken from sites that had been covered by a subdural grid for extended periods of time, signs of acute/subacute inflammation and gliosis were expected and indeed seen in all patients. No definitive clues to epilepsy etiology was seen in any of the tissue samples. The microelectrode tips were positioned in layers 4 and 5 in two patients (Supplementary Figure S2). In one patient (C7), the electrode array was at a slight angle, so that different rows of electrodes sampled different cortical layers, from layer 1 to layer 5. Notably, the activity patterns in this patient, in all electrodes, were qualitatively the same for all analyses. The progression appeared to spread radially across the electrode array (Supplementary movie S5; top is layer 2, bottom is layer 5; left to right no slope), rather than perpendicular to the slope of the electrodes. Furthermore, we saw no evidence of phase reversal suggesting that there was no focal current source; rather the synaptic currents were large in all layers. Together, this suggests strongly that the recruitment of units in all cortical laminae was broadly synchronous, and discounted the possibility that a seizure might invade just a single lamina.