UC Davis
UC Davis Previously Published Works

Title

Quantifying trial-by-trial variability during cortico-cortical evoked potential mapping of epileptogenic tissue.

Permalink

https://escholarship.org/uc/item/2cn6r5hz

Journal

Epilepsia, 64(4)

Authors

Cornblath, Eli

Lucas, Alfredo

Armstrong, Caren

et al.

Publication Date

2023-04-01

DOI

10.1111/epi.17528

Peer reviewed

Quantifying trial-by-trial variability during cortico-cortical evoked potential mapping of epileptogenic tissue

Eli J. Cornblath[1], **Alfredo Lucas**[1,2], **Caren Armstrong**[3], **Adam S. Greenblatt**[1], **Joel M. Stein**[4], **Peter N. Hadar**[1], **Ramya Raghupathi**[1], **Eric Marsh**[1,3,5], **Brian Litt**[1], **Kathryn A. Davis**[#1], **Erin C. Conrad**[#1]

[1]Department of Neurology, Perelman School of Medicine, Philadelphia, Pennsylvania, USA

[2]Department of Bioengineering, School of Engineering & Applied Science, Philadelphia, Pennsylvania, USA

[3]Pediatric Epilepsy Program, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

[4]Department of Radiology, Perelman School of Medicine, Philadelphia, Pennsylvania, USA

[5]Department of Pediatrics, Perelman School of Medicine, Philadelphia, Pennsylvania, USA

[#] These authors contributed equally to this work.

Abstract

Objective: Measuring cortico-cortical evoked potentials (CCEPs) is a promising tool for mapping epileptic networks, but it is not known how variability in brain state and stimulation technique might impact the use of CCEPs for epilepsy localization. We test the hypotheses that (1) CCEPs demonstrate systematic variability across trials and (2) CCEP amplitudes depend on the timing of stimulation with respect to endogenous, low-frequency oscillations.

Methods: We studied 11 patients who underwent CCEP mapping after stereo-electroencephalography electrode implantation for surgical evaluation of drug-resistant epilepsy. Evoked potentials were measured from all electrodes after each pulse of a 30 s, 1 Hz bipolar stimulation train. We quantified monotonic trends, phase dependence, and standard deviation (SD) of N1 (15–50 ms post-stimulation) and N2 (50–300 ms post-stimulation) amplitudes across the 30

stimulation trials for each patient. We used linear regression to quantify the relationship between measures of CCEP variability and the clinical seizure-onset zone (SOZ) or interictal spike rates.

Results: We found that N1 and N2 waveforms exhibited both positive and negative monotonic trends in amplitude across trials. SOZ electrodes and electrodes with high interictal spike rates had lower N1 and N2 amplitudes with higher SD across trials. Monotonic trends of N1 and N2 amplitude were more positive when stimulating from an area with higher interictal spike rate. We also found intermittent synchronization of trial-level N1 amplitude with low-frequency phase in the hippocampus, which did not localize the SOZ.

Significance: These findings suggest that standard approaches for CCEP mapping, which involve computing a trial-averaged response over a .2–1 Hz stimulation train, may be masking inter-trial variability that localizes to epileptogenic tissue. We also found that CCEP N1 amplitudes synchronize with ongoing low-frequency oscillations in the hippocampus. Further targeted experiments are needed to determine whether phase-locked stimulation could have a role in localizing epileptogenic tissue.

1 | INTRODUCTION

Mapping epileptic brain networks and predicting the effects of surgical treatments present major challenges in managing drug-resistant epilepsy (DRE).[1–3] The epileptic network is defined as a set of interacting, distributed brain areas capable of generating seizures.[2] Identifying epileptogenic tissue with electrical stimulation is a promising approach for surgical planning[4–6] compared to provoking seizures, which requires lengthy inpatient stays and continuous monitoring. Measuring cortico-cortical evoked potentials (CCEPs)[6–9] is a common technique for stimulation-based mapping, typically performed intraoperatively or in the epilepsy monitoring unit in patients with intracranial electrode implants. A CCEP is the electrical response observed in one brain area after focal, low-frequency stimulation (<2 Hz) is applied to another brain area. A related technique, known as single-pulse electrical stimulation (SPES), is similar to CCEPs but uses even lower frequency stimulation (1–2 Hz).[7,10] These measurements represent the foundational units of the impulse response of neural networks to the simplest form of electrical perturbation. Studying CCEP responses may lead to improvements in functional mapping as well as in therapies utilizing open loop or responsive neurostimulation.[11]

A fundamental assumption made in many applications of CCEPs[6,12–18] is that the average response over many trials reflects underlying, static effective connectivity. A complementary hypothesis is that inter-trial variability is not random noise that averaging ought to remove, but rather contains important information about brain function and epileptogenicity. Inter-trial variability in CCEPs is an unexplored measurement that may improve functional mapping and stimulation-based therapies for neurologic disorders.

Both qualitative and quantitative metrics can be derived from CCEPs and SPES and have been used extensively to map epileptic networks. We focus on a common quantitative approach, which borrows from the event-related potential (ERP) literature to analyze the amplitude and latency of two stereotypical peaks. The early N1 and late N2 potentials occur 10–50 ms and 50–300 ms post-stimulation, respectively. In general, studies report that N1 amplitude is higher when stimulating and recording from the seizure-onset zone (SOZ),[13,14,19–21] but direct comparison (see Reference (7).[7] for a thorough discussion) between previous findings is hindered by variability across patient populations, hardware, acquisition techniques, post-processing, quantification of N1 and N2, and subsequent analysis. High-frequency oscillations and increased gamma power in the evoked signal[22] can also be found in the SOZ, and qualitatively observed pathological delayed responses localize to resected tissue in patients with post-operative seizure freedom.[4,5] These studies have not examined whether systematic inter-trial variability exists during CCEP mapping, and whether that variability is increased in epileptogenic tissue.

Variability in single-trial CCEP responses may arise from multiple biological mechanisms. Short-term plasticity due to synaptic facilitation,[23] long-term potentiation or long-term depression,[24] and spike-timing dependent plasticity[25] could contribute to variable responses during CCEP mapping, although these phenomena are typically studied at single neuron resolution with higher frequency stimulation (>10 Hz). In addition, theta (4–8 Hz) oscillations are thought to represent fluctuating regional excitability due to phase-locked oscillations in gamma power and neuronal spiking.[26,27] Therefore, the evoked responses may vary systematically, as stimulation occurs at different phases of local or hippocampal theta oscillations. It remains unknown whether these mechanisms underlie inter-trial variability in CCEPs, but further investigation may reveal new insights into epilepsy pathophysiology.

Herein, we analyzed the N1 and N2 amplitudes from 10,032 CCEPs at the single trial level in a cohort of 11 patients with DRE. We test the hypotheses that (1) N1 and N2 amplitudes monotonically increase or decrease across subsequent stimulation trials, and (2) N1 and N2 amplitude depend on the phase of low-frequency oscillations at the hippocampus and recording electrodes at the time of stimulation. We subsequently tested whether these measures of systematic variability, as well as trial-averaged N1 and N2 amplitudes, localize to epileptogenic tissue. In short, we find that monotonic trends in amplitude occur during CCEP mapping, and that N1 and N2 amplitudes are lower but more variable in the SOZ. We found that stimulation responses are related to ongoing hippocampal phase, although this effect was observed only for a small percentage of CCEP stimulation and recording pairs in each patient. These findings introduce new, complementary metrics to CCEP mapping that may improve our ability to localize epileptogenic tissue.

2. | METHODS

2.1 | Participants

This study included 11 patients (Table 1) with DRE admitted to the epilepsy monitoring unit at the Hospital of the University of Pennsylvania. Patients underwent implantation with intracranial stereo-electroencephalography (sEEG) linear depth electrodes (Ad Tech

Medical Instruments). Stimulation was performed at varying times throughout intracranial recording according to clinical availability. In addition, patients were stimulated at different time points in their medication taper. The location of the SOZ was determined by the clinical team evaluating the electroencephalographic onset pattern of clinically habitual seizures. We use the term "epileptogenic tissue" to refer to the SOZ and any other areas with high rates of epileptiform spikes.

2.2 | Electrode localization

In-house software was used to localize electrodes based on pre-and post-implant T1-weighted MPRAGE (magnetization-prepared radio-frequency pulses and rapid gradient-echo) magnetic resonance imaging (MRI) studies, as well as post-implant computed tomography (CT) images. All electrode localizations were verified by a board-certified neuroradiologist (J.M.S.).

We registered all electrode contacts to a three-dimensional (3D) coordinate in Montreal Neurological Institute (MNI) space. We defined white matter electrodes as those whose MNI coordinate was within 1 mm of any voxel in the FMRIB Software Library (FSL) FMRIB58 FA (fractional anisotropy) white matter map,[28] thresholded at a value of 3000.

2.3 | Measurement of cortico-cortical evoked potentials

All implanted depth electrodes had 6–12 cylindrical contacts, each 2.41 mm in length, 1.1 mm in diameter, with 5 mm spacing between adjacent contacts. Sampling rates varied from 512–1024 Hz. Signals were referenced to an electrode distant from the suspected SOZ, usually embedded in medullary bone in the skull. Stimulation was performed while the patient was in the awake relaxed state using a Nicolet Cortical Stimulator (Natus). We delivered bipolar stimulation between adjacent pairs of electrode contacts with the following stimulation parameters: biphasic pulse with 3 mA current, 300 μsec pulse width, 1 Hz stimulation frequency, and 30 s train duration. We attempted to deliver stimulation across either every adjacent pair of contacts or every other adjacent pair of contacts depending on clinical and time constraints (Figure 1). We avoided stimulating any contacts that appeared to have high impedance, lie outside the brain, or were adjacent to the dura (to avoid a painful sensation). We stopped stimulation across a pair of contacts if the patient described any uncomfortable sensation. We stopped the stimulation session entirely if we provoked any seizure.

2.4 | Processing of intracranial EEG data

We extracted CCEP waveforms from intracranial EEG recordings by automatically detecting stimulation artifacts (see Appendix S1 for details). We re-referenced EEG signals to a bipolar montage and averaged signals across all 30 stimulation trials for each pair of stimulation and recording electrode, which we refer to as an average CCEP waveform. Bipolar montage was implemented by subtracting the next higher contact from the one below it on the same electrode. Stimulation trials containing any voltages over 1000 μV were excluded to mitigate the impact of heavy signal artifact. For each average CCEP waveform, we used the MATLAB findpeaks function to find the largest absolute magnitude peak amplitude in the N1 time period (15–50 ms after stimulation) and the N2 time

period (50–300 ms after stimulation). The amplitude of these peaks were *z*-scored against the average baseline EEG from 500 ms to 30 time samples before stimulation.[12,15,16] In addition, we implemented a manually validated artifact rejection approach on the average CCEP waveforms (see Appendix S1 for details). To process CCEPs at the single trial level, we applied a similar pipeline to the EEG data from each trial that also included a 30 Hz low-pass filter, baseline demeaning, and detrending (see Appendix S1 for details), designed to mitigate increased noise with single-trial stimulation. CCEP data obtained during focal aware seizure (FAS) for HUP224 were discarded, but data obtained during FAS for HUP225 were included because the electrographic seizure did not begin until after the 30th and final trial. Otherwise, no ictal EEG data were used in this study.

2.5 | Quantification of dynamic responses to brain stimulation

To test our hypothesis that N1 and N2 waveforms exhibit monotonic trends, we computed the Spearman rank correlation (ρ) between the stimulation trial index (1–30) and the N1 or N2 waveform amplitude for each trial. This correlation was computed for every stimulation and recording electrode pair for which a suprathreshold CCEP was obtained. Stimulation trials rejected for artifact were excluded and ignored when computing this correlation. The *p*-values from these correlations were adjusted for multiple comparisons by false discovery rate (FDR) correction[29] across all the CCEPs separately for each patient.

We applied a similar approach to test our hypothesis that N1 and N2 waveform amplitudes were related to the pre-stimulation phase of low-frequency oscillations. In brief, we computed the circular-linear correlation[30] between N1 or N2 waveforms and the estimated the low-frequency phase (see Appendix S1) at the time of stimulation in either the hippocampus or the recording electrode for each CCEP. We adjusted all *p*-values to an FDR of $q < .05$ across all the CCEPs within each patient, separately for each hippocampal electrode.

3. | RESULTS

3.1 | N1 and N2 amplitudes show monotonic trends across trials

We began our study by testing our hypothesis that N1 and N2 waveform amplitudes monotonically increase or decrease over the course of a 30 s, 1 Hz stimulation train. We computed the Spearman rank correlation between trial number and the amplitude of the N1 and N2 waveform for each detectable CCEP. We found many CCEPs with statistically significant positive or negative Spearman correlations at $p_{FDR} < .05$, for both N1 and N2 waveforms (see Figure 2 for examples of individual CCEPs; Figure 3A, left, for distribution). These statistically significant monotonic trends were found across a range of inter-electrode distances (Figure 3A, right). We found statistically significant monotonic trends in all but 1 of 11 patients (HUP211), likely due to the small number of stimulating electrodes tested (Figure 3B). Within each patient (Table S1), these effects occurred in .84% of all supra-threshold CCEPs on average for N1 (range of 0%–3.20%) and in .94% of all supra-threshold CCEPs on average for N2 (range of 0%–3.58%). Of interest, of the 17 CCEPs with statistically significant N1 monotonic trends for HUP 225, 11 of them occurred while stimulating electrode RC1 (hippocampus). After the 30th and final

stimulation trial, the patient had a typical focal aware seizure (Figure S11; see Figure 2C for representative CCEP obtained during seizure). These findings suggest that there are infrequent, bidirectional monotonic trends of N1 and N2 amplitude across trials during CCEP mapping. Next, we assessed whether positive and negative trends were mutually exclusive or co-occurred at the same electrodes. Within each patient, we found at least one CCEP with a statistically significant monotonic trend in 4.89% of recording electrodes with suprathreshold CCEPs on average for N1 (range of 0%–17.02%) and in 5.90% of recording electrodes with suprathreshold CCEPs on average for N2 (range of 0%–27.27%). We rarely found both positive and negative statistically significant trends at the same recording electrode within the same patient (Figure 3B). We saw more overlap between positive and negative trends within the same anatomic parcels in the Brainnetome atlas,[31] but the majority of effects were consistent within patients (Figure S5). However, we did see both positive and negative statistically significant trends within the same anatomic areas across patients (Figure S5). The temporal and anatomic consistency of these trends is difficult to assess given the limited sampling of the brain with sEEG and the short stimulation trains.

3.2 | Relationship between low-frequency oscillations and stimulation responses

In a second set of experiments, we tested the hypothesis that the phase of ongoing low-frequency brain oscillations mediates the brain's response to direct electrical stimulation. Theta-range oscillations in the hippocampus have been shown to correlate with spiking activity in prefrontal cortex,[27] and theta-gamma cross-frequency coupling within brain areas has been observed in human electrophysiologic data across a range of cognitive tasks as well as the resting state.[26] Therefore, we hypothesized that the low-frequency phase at the hippocampus and at recording electrodes would correlate with N1 and N2 amplitude evoked by stimulation during individual trials (see Appendix S1, "Quantifying low frequency oscillations" for details). We computed the circular-linear correlation[30] between N1 or N2 amplitude (linear) at each trial and phase (circular) at either hippocampal electrodes (Figure 4B) or recording electrodes (Figure 4C) at the time of stimulation for each CCEP trial. For the hippocampal electrode phase, within each patient, these effects occurred in .80% of all supra-threshold CCEPs on average for N1 (range of 0%–5.79%) and in .12% of all supra-threshold CCEPs on average for N2 (range of 0%–.87%). The statistically significant correlations ranged from .62 to .90 (variance explained 38%–80%). These findings suggest that the N1 amplitude depends on the hippocampal signal phase during CCEP mapping, although this effect occurs infrequently.

3.3 | Overlap between CCEP measures and epileptogenic tissue

After identifying statistically significant monotonic trends in CCEP amplitudes, we next sought to test whether inter-trial variability preferentially occurred in epileptogenic tissue, as defined by the clinically determined SOZ and rates of interictal spiking at each electrode contact (see Appendix S1, "Epileptic spike detection"). To test this hypothesis, we fit linear regression models for each patient, with covariates controlling for inter-electrode distance and localization of stimulation and recording electrodes to gray matter, white matter, primary motor cortex, primary sensory cortex, and the occipital lobe (see Appendix S1 for formulation). In addition to the strength of monotonic trends across trials (Spearman ρ from Figure 3), we also quantified the standard deviation (SD) of N1 or N2 amplitude

across trials. All regression statistics (model *F* statistic, degrees of freedom, coefficient values, coefficient standard errors, *p*-values, and p_{FDR} values) for each patient's linear model for each dependent variable can be found in Data S1, but are omitted in the text due to the large number of values (10 patients with up to 12 linear models each in Figure 5A–F). *p*-values for each regression coefficient were FDR corrected across all patients separately for each linear model formulation, corresponding to each panel of Figure 5.

Contrary to previous studies, we found that CCEPs involving SOZ electrodes (Inside SOZ) showed lower amplitude N1 and N2 waveforms compared to CCEP waveforms recorded from stimulation and recording electrodes lying solely outside of the SOZ (Outside SOZ) (Figure 5A). However, this effect was statistically significant for only three patients for N1 and three patients for N2. Next we found that the strength of monotonic trend did not strongly localize to the SOZ, and was only significantly increased in the SOZ for one patient (Figure 5B). Finally, we found that the SD of N1 and N2 amplitudes across trials tended to be higher in the SOZ than outside of the SOZ (Figure 5C). This relationship was statistically significant for two patients for N1 and three patients for N2 (Figure 5C) and directionally consistent for an additional two patients. Notably, across all CCEPs, N1 amplitude is generally not related to N1 amplitude variability, whereas N2 amplitude is positively related to N2 amplitude variability (see Figure S9 and Appendix S1, subsection "Relationship between CCEP amplitude and CCEP amplitude variability"). In Table S2, we report the odds ratios for each of these CCEP metrics in estimating the probability that a CCEP belongs to the SOZ using a modified formulation of the models described above (see Appendix S1). Notably, the effect size is small (increase in odds of about 10%–20%) for a 1 SD change in CCEP amplitude or inter-trial variability metrics. Overall, these findings suggest that for a subset of patients, CCEPs in the SOZ may be lower in amplitude and more variable across trials relative to CCEPs outside of the SOZ.

In addition to examining the clinical SOZ, we also examined whether trial-averaged CCEP amplitudes, monotonic trend strength, and SD of CCEP responses covaried with the rate of interictal spiking at each electrode. Here, we separately tested whether CCEP metrics were related to spiking at recording or stimulating electrodes. Consistent with our finding of reduced N1 and N2 amplitudes in the SOZ (Figure 5A), we found statistically significant negative relationships between N1 and N2 amplitudes and spiking at both stimulating and recording electrodes (Figure 5D). We found mostly positive relationships between monotonic trend strength (Fisher *r*-to-*z*-transformed Spearman ρ) and spike rates at the stimulating electrode (Figure 5E), an effect that was stronger for N1 amplitude. Finally, we found that the SD of N1 and N2 amplitudes was positively related to spike rates at both the recording and stimulating electrodes (Figure 5F). Standardized logistic regression β weights shown in Figures S7 and S8 approximate the square root of the variance in epileptic spike rates explained by each variable for each patient. We did not find any relationship between hippocampal phase dependence (Figure 4B) and spike rates or SOZ location (Figure S10). Taken together, these findings suggest that areas with higher spike rates tend to have lower amplitude and more variable CCEPs, and stimulating electrodes with higher spike rates tend to elicit more positive monotonic trends across trials.

4 | DISCUSSION

In this article, we introduce a framework for quantifying trial-by-trial variability during CCEP mapping and relating it to epileptogenic tissue. We found that several CCEPs show positive or negative monotonic trends in N1 and N2 amplitudes across trials. We also found that the N1 amplitude is related to low-frequency phase in the hippocampus across trials for a small percentage of stimulating and recording electrode pairs in 7 of 11 patients. We investigated the relationship between CCEPs and epileptogenic tissue and found that areas in the SOZ or with more interictal spikes tend to have lower amplitude N1 and N2 with higher SDs across trials. These findings introduce a new, complementary approach for quantifying CCEPs, which require further validation in larger samples before they can be used for probing pathologic brain function in epilepsy.

4.1 | Implications for CCEP mapping of epileptogenic tissue

Our study expands on a large body of existing literature on CCEP and SPES mapping for localizing epileptic networks[13,14,19–21,32] and identifying targets for surgical resection.[4,5] We contribute to this literature with two new findings. First, we directly relate CCEP responses to quantitative interictal spike rates, which have not been studied previously to our knowledge. Using this approach, we find increased interictal spike rates in regions with more positive monotonic trends, suggesting that increasing response amplitude over a low-frequency stimulation train may measure abnormalities that give rise to epileptiform activity. Supporting that observation, we observed 11 CCEPs with positive monotonic trends during a stimulation train that was followed by a typical focal aware seizure in HUP225. Second, we find that CCEP amplitudes are lower and more variable across trials in the SOZ and areas with high spike rates. N2 amplitude was positively related to SD across trials, so the combination of lower CCEP amplitudes with higher variability would not be expected and may be a unique phenomenon in epileptogenic tissue. Of interest, these effects were found in patients with unilateral mesial temporal lobe epilepsy (mTLE), bilateral mTLE, and neocortical TLE. These findings provide preliminary evidence for the utility of studying inter-trial variability in CCEPs in addition to current approaches.

We also find that N1 and N2 average amplitudes are lower in the SOZ and in areas with higher spike rates. We observed statistically significant trends for 6 of 10 patients, but nearly all patients showed a directionally consistent effect. This finding was unexpected based on previous studies,[13,14,19–21] which mostly find that N1 amplitude is increased in the SOZ. One reason for this discrepancy could be our stringent artifact rejection and spatially clustered SOZ electrodes, which may have led to systematic rejection of CCEPs recording in the SOZ from SOZ stimulating electrodes. In fact, we recorded only eight of such CCEPs passing artifact rejection for both N1 and N2 peaks. Nevertheless, our analysis of interictal spike rates also revealed the same negative relationship while providing a more continuous measure of epileptiform activity than the binary SOZ electrode mask. Finally, we also control for inter-electrode distance, which is related negatively to CCEP amplitude, so other studies may be conflating the effect of the SOZ with the effect of spatial clustering.

In addition, some studies compared a single non-SOZ control stimulation area to an SOZ area,[13,19] whereas we broadly sampled areas outside the SOZ, which may have contributed

to the finding of low CCEP amplitude in the SOZ (Figure S13). Physiologic inter-regional differences in CCEP amplitude may exceed the difference between a normal region and epileptic tissue in that same region, thereby confounding the effect of the SOZ on CCEP amplitudes. Normative modeling and atlas-based approaches have been applied to EEG data to overcome this issue,[33] but have yet to reach the field of CCEP analysis. Finally, although most previous studies present group-level statistics,[13,14,19–21] we fit unique models to each patient and found that CCEPs localize the SOZ only for some patients. Further model development is needed to overcome the limitations of this significant inter-individual variability while allowing for generalization to new patients for out-of-sample prediction. The models studied here provide "training sample" estimates useful for inference only[34] and do not quantify performance at SOZ localization from CCEP data alone in a new patient admitted to the epilepsy monitoring unit. Therefore, analyzing group effects may uncover significant inter-individual variability that is critical to understand when using quantitative CCEP metrics clinically. Finally, a more practical use of CCEPs might involve distinguishing between two surgical hypotheses, rather than broadly identifying the SOZ, although the latter exercise is an important proof of concept.

4.2 | Methodologic considerations in analyzing CCEPs data

In attempting to compare our findings to the existing literature, we noted significant variability in approaches for acquiring, processing, and analyzing CCEPs data. Some studies utilize subdural electrocorticography (ECoG) grids[12,16,19] whereas others, including ours, use stereoelectroencephalography (sEEG) depth electrodes.[14,15,20] This hardware difference impacts seizure localization from interictal data,[35] but it has not yet been systematically studied in CCEPs. Quantification of N1 and N2 amplitudes is highly variable as well. Some studies use the root mean square of the signal,[14,20,36] others use global maximum amplitude signal[12,15,16] or spectral power,[37] and other studies, including ours, identify local maxima to directly measure peak amplitude.[13,19,21] Methods for spectral filtering of CCEP data vary significantly in the literature,[7] although in the present study, our findings were similar when we applied a preprocessing pipeline without a 30 Hz low-pass filter (see Appendix S1, "Sensitivity of findings to filter settings"). After quantifying CCEP responses, our study and one other used linear regression to adjust for distance and tissue type,[20] whereas most others directly compare epileptic and nonepileptic tissue without adjusting for these potential confounders. The impact of all of these methodologic choices on seizure localization warrants further study in a larger data set in order to improve the generalizability and reliability of stimulation-based mapping of epileptic networks.

4.3 | Biological underpinnings of inter-trial variability

Although we do not provide experimental evidence supporting a particular mechanism for the monotonic trends of N1 and N2 amplitude we observed, our data are not consistent with any known type of synaptic plasticity. Short-term synaptic facilitation and depression occur on the order of milliseconds at higher stimulation frequencies (>10 Hz).[23] Long-term potentiation classically occurs with high-frequency stimulation and long-term depression occurs with low-frequency stimulation,[38] but we see both positive and negative correlations in CCEP amplitudes across trials. It is possible that an unmeasured, local, slow oscillation near .01 Hz could coincide with the entire stimulation train and induce both positive

and negative trends depending on the phase. We controlled for signal drift by baseline demeaning and detrending, so the effects of such an oscillation would have to act directly on the stimulation response to produce this effect. Finally, we saw a relationship between CCEP amplitudes and hippocampal low-frequency signal phase for only a small percentage of all measured CCEPs. Ideally, we would have measured the phase at the stimulating electrode, but we were unable to do so because of amplifier saturation near the stimulation electrode. Regardless, 30 stimulation pulses may not be sufficient to sample the phase of ongoing oscillations to detect systematic effects on stimulation response. Targeted experiments with a larger number of stimulation trials and phase-locked stimulation are needed to sufficiently test this hypothesis.

4.4 | Methodological limitations

As discussed previously, spatial sampling of the brain was constrained by clinical indication and safety of electrode placement. Stimulation artifact limited our ability to assess early responses (<15 ms post-stimulation) in the CCEP waveform. We also exclusively used 3 mA stimulation in order to minimize tissue damage; however, prior studies[13,19,20,39] have found that differences between SOZ and non-SOZ CCEPs begin at 3 mA and are maximal at 6–8 mA. In addition, amplifier saturation near the stimulating electrodes prevented us from quantifying low-frequency oscillations at the stimulation electrode. The small number of patients studied here contained mostly mesial temporal lobe epilepsy, and, therefore, may not generalize to neocortical epilepsies. Despite the small number of patients, we were able to record a large number of CCEPs (10 032 total) and we present data analyzed at the single patient level. Finally, it was not feasible to control for epileptic spikes occurring during the stimulation session due to lack of a well-validated detector that distinguishes physiologic CCEPs from epileptic spikes. Therefore, it is possible that epileptic spikes or "delayed responses"[4,5] may have contributed to the finding of low-amplitude, high-variability CCEPs in epileptogenic tissue.

4.5 | Future directions

These findings naturally lead to several future experiments and analyses aimed at exploring the mechanisms of inter-trial variability and improving the accuracy of CCEP mapping of epileptic networks. First, one could perform a longer 1 Hz stimulation train to observe whether these monotonic trends continue or die off over time. Second, one could deliver pulses synchronized to peaks and troughs of low frequency oscillations with the aim of creating a measure of normal brain physiology that might distinguish epileptogenic from healthy tissue. Finally, one could attempt to aggregate a large number of CCEPs data sets with annotated SOZs, surgical outcomes, and quantitative resection masks. Such a data set would allow for rigorous benchmarking of methods against ultimate SOZ localization, development of a normative CCEP atlas, and model selection for the prediction of surgical outcomes and SOZ localization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

CITATION DIVERSITY STATEMENT

Recent work in several fields of science has identified a bias in citation practices such that papers from women and other minority scholars are under-cited relative to the number of such papers in the field.[40–48] Here we sought to proactively consider choosing references that reflect the diversity of the field in thought, form of contribution, gender, race, ethnicity, and other factors. First, we obtained the predicted gender of the first and last author of each reference by using databases that store the probability of a first name being carried by a woman.[44,49] By this measure (and excluding self-citations to the first and last authors of our current paper), our references contain 13.82% woman(first)/woman(last), 8.71% man/woman, 17.66% woman/man, and 59.81% man/man. This method is limited in that (1) names, pronouns, and social media profiles used to construct the databases may not, in every case, be indicative of gender identity and (2) it cannot account for intersex, non-binary, or transgender people. Second, we obtained predicted racial/ethnic category of the first and last author of each reference by databases that store the probability of a first and last name being carried by an author of color.[50,51] By this measure (and excluding self-citations), our references contain 18.66% author of color (first)/author of color(last), 19.11% white author/author of color, 14.44% author of color/white author, and 47.8% white author/white author. This method is limited in that (1) names and Florida Voter Data to make the predictions may not be indicative of racial/ethnic identity, and (2) it cannot account for indigenous and mixed-race authors, or those who may face differential biases due to the ambiguous racialization or ethnicization of their names. We look forward to future work that could help us to better understand how to support equitable practices in science.

REFERENCES.

1. Jehi L. Algorithms in clinical epilepsy practice: can they really help us predict epilepsy outcomes? Epilepsia. 2021;62:S71–7. 10.1111/EPI.16649 [PubMed: 32871035]

2. Davis KA, Jirsa VK, Schevon CA. Wheels within wheels: theory and practice of epileptic networks. Epilepsy Curr. 2021;21:15357597211015663. 10.1177/15357597211015663 [PubMed: 33988042]

3. Zijlmans M, Zweiphenning W, van Klink N. Changing concepts in presurgical assessment for epilepsy surgery. Nat Rev Neurol. 2019;15:594–606. 10.1038/s41582-019-0224-y [PubMed: 31341275]

4. Valent'ın A, Alarcon G, Honavar M, Garc'ıa Seoane JJ, Selway RP, Polkey CE, et al. . Single pulse electrical stimulation for identification of structural abnormalities and prediction of seizure

outcome after epilepsy surgery: a prospective study. Lancet Neurol. 2005;4:718–26. 10.1016/S1474-4422(05)70200-3 [PubMed: 16239178]

5. Valentin A, Alarcon G. Single pulse electrical stimulation in presurgical assessment of epilepsy: a new diagnostic tool. Adv Clin Neurosci Rehabil. 2008;8:14–8.

6. Matsumoto R, Kunieda T, Nair D. Single pulse electrical stimulation to probe functional and pathological connectivity in epilepsy. Seizure. 2017;44:27–36. 10.1016/J.SEIZURE.2016.11.003 [PubMed: 27939100]

7. Prime D, Rowlands D, O'Keefe S, Dionisio S. Considerations in performing and analyzing the responses of cortico-cortical evoked potentials in stereo-EEG. Epilepsia. 2018;59:16–26. 10.1111/EPI.13939 [PubMed: 29143307]

8. Keller CJ, Honey CJ, M'egevand P, Entz L, Ulbert I, Mehta AD. Mapping human brain networks with cortico–cortical evoked potentials. Philos Trans R Soc Lond B Biol Sci. 2014;369. 10.1098/rstb.2013.0528

9. Vincent M, Guiraud D, Duffau H, Mandonnet E, Bonnetblanc F, Bonnetblanc Elec F. Electrophysiological brain mapping: basics of recording evoked potentials induced by electrical stimulation and its physiological spreading in the human brain. Clin Neurophysiol. 2017;128:1886–90. 10.1016/j.clinph.2017.07.402 [PubMed: 28826018]

10. Valent'ın A, Anderson M, Alarc'on G, Garc'ıa Seoane JJ, Selway R, Binnie CD, et al. Responses to single pulse electrical stimulation identify epileptogenesis in the human brain in vivo. Brain. 2002;125:1709–18. 10.1093/BRAIN/AWF187 [PubMed: 12135963]

11. Thomas GP, Jobst BC. Critical review of the responsive neurostimulator system for epilepsy. Med Devices (Auckl). 2015;8:405. 10.2147/MDER.S62853 [PubMed: 26491376]

12. Keller CJ, Bickel S, Entz L, Ulbert I, Milham MP, Kelly C, et al. Intrinsic functional architecture predicts electrically evoked responses in the human brain. Proc Natl Acad Sci U S A. 2011;108:10308–13. 10.1073/pnas.1019750108 [PubMed: 21636787]

13. Iwasaki M, Enatsu R, Matsumoto R, Novak E, Thankappen B, Piao Z, et al. Accentuated cortico-cortical evoked potentials in neocortical epilepsy in areas of ictal onset. Epileptic Disord. 2010;12:292–302. 10.1684/epd.2010.0334 [PubMed: 20952353]

14. hao Guo Z, tian Zhao B, Toprani S, han Hu W, Zhang C, Wang X, et al. Epileptogenic network of focal epilepsies mapped with cortico-cortical evoked potentials. Clin Neurophysiol. 2020;131:2657–66. 10.1016/J.CLINPH.2020.08.012 [PubMed: 32957038]

15. Crocker B, Ostrowski L, Williams ZM, Dougherty DD, Eskandar EN, Widge AS, et al. Local and distant responses to single pulse electrical stimulation reflect different forms of connectivity. Neuroimage. 2021;237:118094. 10.1016/J.NEUROIMAGE.2021.118094 [PubMed: 33940142]

16. Keller CJ, Honey CJ, Entz L, Bickel S, Groppe DM, Toth E, et al. Corticocortical evoked potentials reveal projectors and integrators in human brain networks. J Neurosci. 2014;34:9152–63. 10.1523/JNEUROSCI.4289-13.2014 [PubMed: 24990935]

17. Kamali G, Smith RJ, Hays M, Coogan C, Crone NE, Kang JY, et al. Transfer function models for the localization of seizure onset zone from Cortico-cortical evoked potentials. Front Neurol. 2020;11:1431. 10.3389/FNEUR.2020.579961/BIBTEX

18. Mariani V, Sartori I, Revay M, Mai R, Lizio D, Berta L, et al. Intraoperative Corticocortical evoked potentials for language monitoring in epilepsy surgery. World Neurosurg. 2021;151:e109–21. 10.1016/J.WNEU.2021.03.141 [PubMed: 33819704]

19. Enatsu R, Piao Z, O'Connor T, Horning K, Mosher J, Burgess R, et al. Cortical excitability varies upon ictal onset patterns in neocortical epilepsy: a cortico-cortical evoked potential study. Clin Neurophysiol. 2012;123:252–60. 10.1016/J.CLINPH.2011.06.030 [PubMed: 21802356]

20. Kundu B, Davis TS, Philip B, Smith EH, Arain A, Peters A, et al. A systematic exploration of parameters affecting evoked intracranial potentials in patients with epilepsy. Brain Stimul. 2020;13:1232–44. 10.1016/J.BRS.2020.06.002 [PubMed: 32504827]

21. Zhao C, Liang Y, Li C, Gao R, Wei J, Zuo R, et al. Localization of Epileptogenic zone based on Cortico-cortical evoked potential (CCEP): a feature extraction and graph theory approach. Front Neuroinform. 2019;13:31. 10.3389/fninf.2019.00031 [PubMed: 31068798]

22. Van'T Klooster MA, Zijlmans M, Leijten FS, Ferrier CH, Van Putten MJ, Huiskamp GJ. Time–frequency analysis of single pulse electrical stimulation to assist delineation of epileptogenic cortex. Brain. 2011;134:2855–66. 10.1093/BRAIN/AWR211 [PubMed: 21900209]

23. Jackman SL, Regehr WG. The mechanisms and functions of synaptic facilitation. Neuron. 2017;94:447–64. 10.1016/J.NEURON.2017.02.047 [PubMed: 28472650]

24. Malenka RC, Nicoll RA. Long-term potentiation -a decade of progress? Science. 1999;285:1870–4. 10.1126/SCIENCE.285.5435.1870/ASSET/E563D395-8804-4A47-81FE-404D9C80F490/ASSETS/GRAPHIC/SE3797846002.JPEG. [PubMed: 10489359]

25. Abbott LF, Nelson SB. Synaptic plasticity: taming the beast. Nat Neurosci. 2000;3:1178–83. 10.1038/81453 [PubMed: 11127835]

26. Lisman JE, Jensen O. The theta-gamma neural code. Neuron. 2013;77:1002–16. 10.1016/J.NEURON.2013.03.007 [PubMed: 23522038]

27. Siapas AG, Lubenov EV, Wilson MA. Prefrontal phase locking to hippocampal theta oscillations. Neuron. 2005;46:141–51. 10.1016/J.NEURON.2005.02.028 [PubMed: 15820700]

28. Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. FSL. Neuroimage. 2012;62:782–90. 10.1016/j.neuroimage.2011.09.015 [PubMed: 21979382]

29. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B Methodol. 1995;57:289–300.

30. Berens P. CircStat: a MATLAB toolbox for circular statistics. J Stat Softw. 2009;31:1–21. 10.18637/JSS.V031.I10

31. Fan L, Li H, Zhuo J, Zhang Y, Wang J, Chen L, et al. The human Brainnetome atlas: a new brain atlas based on connectional architecture. Cereb Cortex. 2016;26:3508–26. 10.1093/CERCOR/BHW157 [PubMed: 27230218]

32. Zhang N, Zhang B, Rajah GB, Geng X, Singh R, Yang Y, et al. The effectiveness of cortico-cortical evoked potential in detecting seizure onset zones. Neurol Res. 2018;40:480–90. 10.1080/01616412.2018.1454092 [PubMed: 29575990]

33. Bernabei JM, Sinha N, Arnold TC, Conrad E, Ong I, Pattnaik AR, et al. Normative intracranial EEG maps epileptogenic tissues in focal epilepsy. Brain. 2022;145:1949–61. 10.1093/BRAIN/AWAB480 [PubMed: 35640886]

34. Poldrack RA, Huckins G, Varoquaux G. Establishment of best practices for evidence for prediction a review. JAMA Psychiat. 2020;77:534–40. 10.1001/JAMAPSYCHIATRY.2019.3671

35. Bernabei JM, Arnold TC, Shah P, Revell A, Ong IZ, Kini LG, et al. Electrocorticography and stereo EEG provide distinct measures of brain connectivity: implications for network models. Brain Commun. 2021;3:1–11. 10.1093/BRAINCOMMS/FCAB156

36. Boido D, Kapetis D, Gnatkovsky V, Pastori C, Galbardi B, Sartori I, et al. Stimulus-evoked potentials contribute to map the epileptogenic zone during stereo-EEG presurgical monitoring. Hum Brain Mapp. 2014;35:4267–81. 10.1002/HBM.22516 [PubMed: 24706574]

37. Trebaul L, Rudrauf D, Job AS, Mˆıia MD, Popa I, Barborica A, et al. Stimulation artifact correction method for estimation of early cortico-cortical evoked potentials. J Neurosci Methods. 2016;264:94–102. 10.1016/J.JNEUMETH.2016.03.002 [PubMed: 26952846]

38. Lü Scher C, Malenka RC, Sheng M, Sabatini B, Sü TC. NMDA receptor-dependent long-t erm Potentiation and long-t erm depression (LTP/LTD). Cold Spring Harb Perspect Biol. 2012;4:a005710. 10.1101/CSHPERSPECT.A005710 [PubMed: 22510460]

39. Hays MA, Smith RJ, Wang Y, Coogan C, Sarma SV, Crone NE, et al. Cortico-cortical evoked potentials in response to varying stimulation intensity improves seizure localization. Clin Neurophysiol. 2022;145:119–28. 10.1016/J.CLINPH.2022.08.024 [PubMed: 36127246]

40. Mitchell SM, Lange S, Brus H. Gendered citation patterns in international relations journals. Int Stud Perspect. 2013;14:485–92.

41. Dion ML, Sumner JL, Mitchell SML. Gendered citation patterns across political science and social science methodology fields. Polit Anal. 2018;26:312–27. 10.1017/pan.2018.12

42. Caplar N, Tacchella S, Birrer S. Quantitative evaluation of gender bias in astronomical publications from citation counts. Nat Astron. 2017;1:1–5. 10.1038/s41550-017-0141

43. Maliniak D, Powers R, Walter BF. The gender citation gap in international relations. Int Organ. 2013;67:889–922. 10.1017/S0020818313000209

44. Dworkin JD, Linn KA, Teich EG, Zurn P, Shinohara RT, and Bassett DS: The extent and drivers of gender imbalance in neuroscience reference lists. bioRxiv. 2020. 2020.01.03.894378. 10.1101/2020.01.03.894378.

45. Bertolero MA, Dworkin JD, David SU, Lloreda CL, Srivastava P, Stiso J, Zhou D, Dzirasa K, Fair DA, Kaczkurkin AN et al. : Racial and ethnic imbalance in neuroscience reference lists and intersections with gender. bioRxiv 2020.

46. Wang X, Dworkin JD, Zhou D, Stiso J, Falk EB, Bassett DS, et al. Gendered citation practices in the field of communication. Ann Int Commun Assoc. 2021;45:134–53. 10.1080/23808985.2021.1960180 [PubMed: 34541322]

47. Chatterjee P, Werner RM. Gender disparity in citations in high-impact journal articles. JAMA Netw Open. 2021;4:e2114509. [PubMed: 34213560]

48. Fulvio JM, Akinnola I, Postle BR. Gender (Im)balance in citation practices in cognitive neuroscience. J Cogn Neurosci. 2021;33:3–7. [PubMed: 33078992]

49. Zhou D, Cornblath EJ, Stiso J, Teich EG, Dworkin JD, Blevins AS, and Bassett DS: Gender Diversity Statement and Code Notebook v1.0 2020. 10.5281/ZENODO.3672110.

50. Ambekar A, Ward C, Mohammed J, Male S, and Skiena S: Name-ethnicity classification from open sources. In Proceedings of the 15th ACM SIGKDD international conference on knowledge discovery and data mining. 2009 49–58.

51. Sood G and Laohaprapanon S: Predicting race and ethnicity from the sequence of characters in a name. arXiv Preprint arXiv:180502109.2018.

Key points

- We observed positive and negative monotonic trends in N1 and N2 amplitudes across a 30 s, 1 Hz stimulation train during cortico-cortical evoked potential (CCEP) mapping.

- We found that for some CCEPs, N1 amplitude synchronizes with the phase of low-frequency hippocampal oscillations at the time of stimulation.

- We found that the seizure-onset zone and areas with higher interictal spike rates had lower N1 and N2 amplitudes with higher standard deviations across trials.
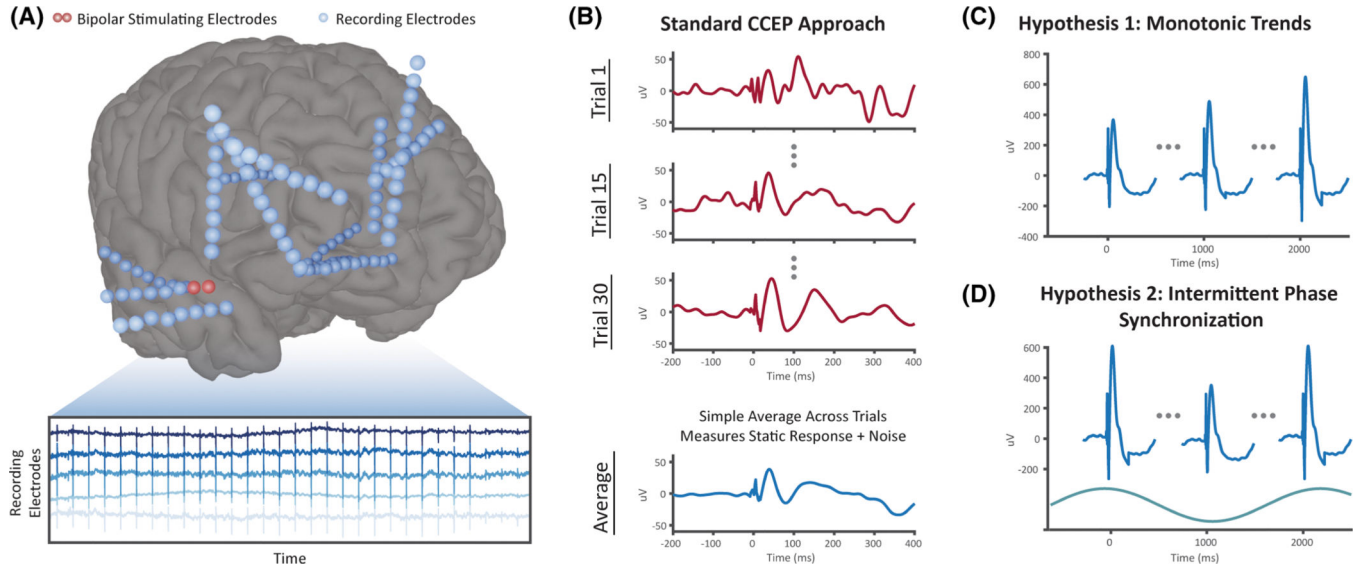
FIGURE 1.

Measuring cortico-cortical evoked potentials (CCEPs) and testing for dynamic responses. (A) We measured CCEPs in a cohort of patients admitted to the epilepsy monitoring unit for surgical evaluation of drug-resistant epilepsy. Thirty seconds of 1 Hz bipolar electrical stimulation was applied to adjacent contacts on stereo-electroencephalography (sEEG) depth electrodes, and evoked potentials were recorded from all electrodes. (B) The standard approach for measuring CCEPs only measures a constant response across trials by averaging all stimulation trials to obtain one single representative CCEP waveform for each stimulation-recording pair. Therefore, the standard approach is agnostic to any trends in amplitude across trials. (C, D) Here, we test two alternative hypotheses. First (C), we hypothesize that monotonic trends may occur, such that response amplitudes increase or decrease monotonically across subsequent stimulation trials. Second (D), we hypothesize that response amplitudes covary with the phase and amplitude of ongoing low-frequency oscillations due to fluctuations in cortical excitability.
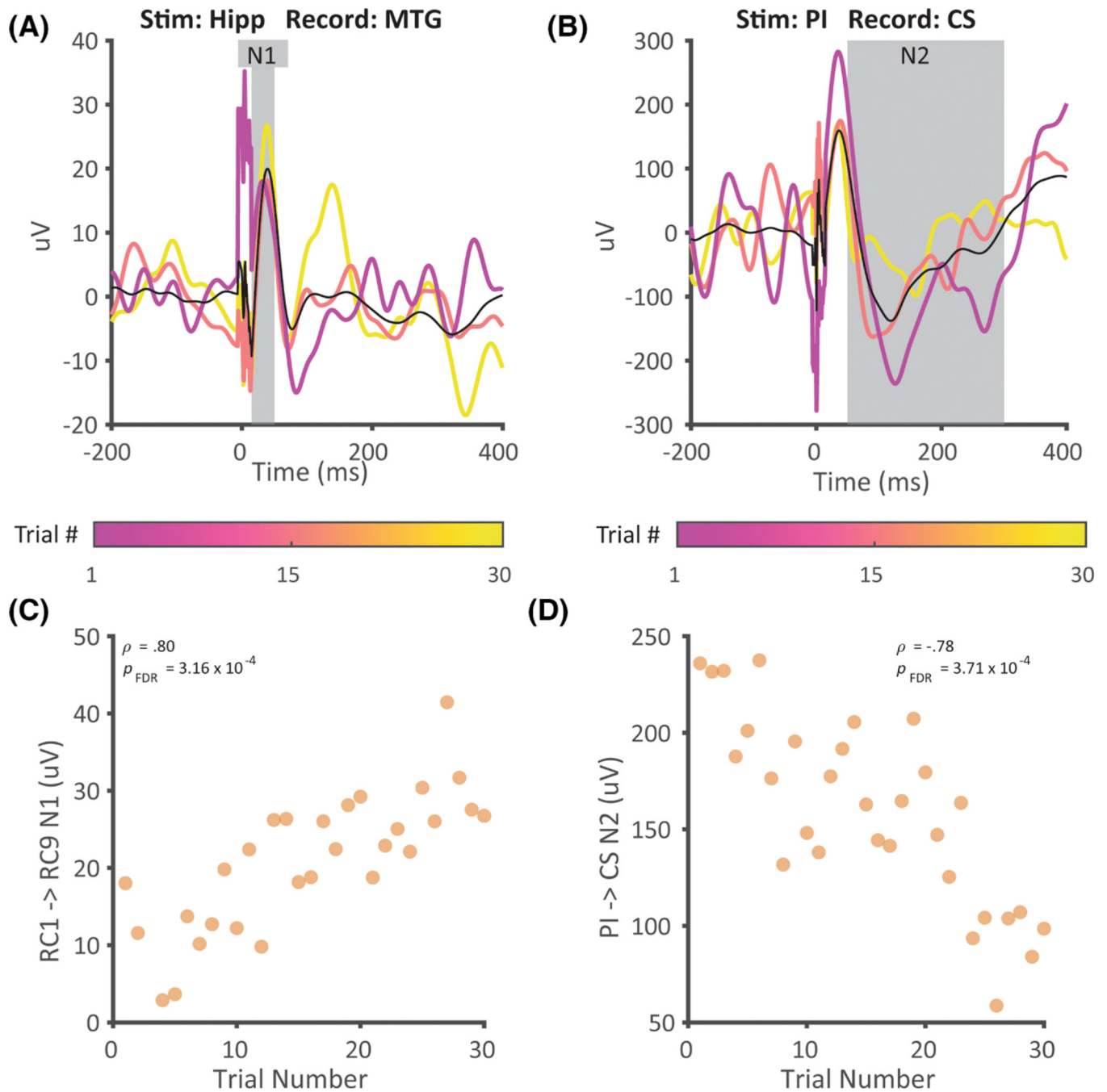
FIGURE 2.

Representative cortico-cortical evoked potentials (CCEPs) demonstrating monotonic trends. (A, B) Examples of CCEP waveforms with statistically significant monotonic trends across trials. Average waveform is plotted in black, and individual trials are plotted with line color corresponding to the trial index. The N1 (A) and N2 (B) periods are highlighted in gray. (C, D) The absolute value of the N1 (C) and N2 (D) amplitudes (*y*-axis) are plotted against trial index (*x*-axis) within the 30 s, 1 Hz stimulation train. Spearman rank correlations are shown and *p*-values were adjusted to a false discovery rate (FDR) $q < .05$ across all

detectable CCEPs separately for each patient. Hipp, hippocampus. MTG, middle temporal gyrus. PI, posterior insula. CS, central sulcus. Of interest, acquisition of the CCEP shown in C was followed by a typical focal aware seizure in HUP225 (see Section 3 for details). Electrophysiologic data shown in this figure are bipolar referenced, detrended, and low-pass filtered at 30 Hz, with a sampling rate of 1024 Hz.
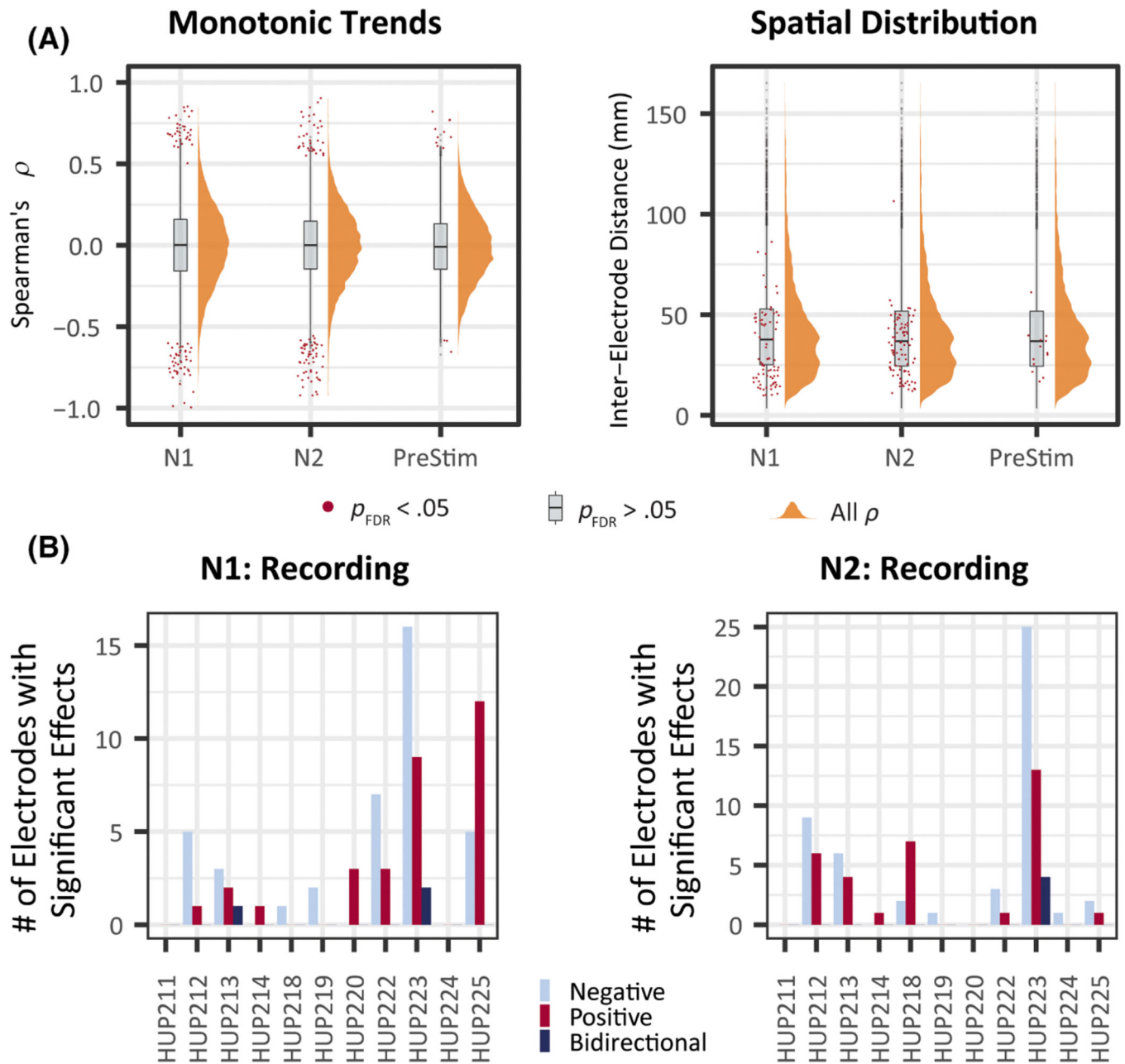
FIGURE 3.

Monotonic trends across trials in cortico-cortical evoked potential (CCEP) amplitudes. (A) In the left panel, we quantified monotonic trends in CCEP amplitudes by plotting distributions of Spearman rank correlations between trial index and either N1 amplitude, N2 amplitude, or amplitude during the −300 to −200 ms period relative to stimulation ("PreStim") as a negative control stratified by pFDR < .05. In the right panel, we plotted the inter-electrode distances for each CCEP stratified by statistical significance of the Spearman correlation between trial index and waveform amplitude. All correlations or distances are plotted in the orange histogram, correlations with pFDR < .05 are plotted as red dots, and correlations with pFDR > .05 are shown as a gray boxplot. (B) The number of

recording electrodes for each patient with CCEPs showing statistically significant monotonic trends, stratified by whether both positive and negative trends occur at the same electrode (bidirectional) vs only positive or negative.
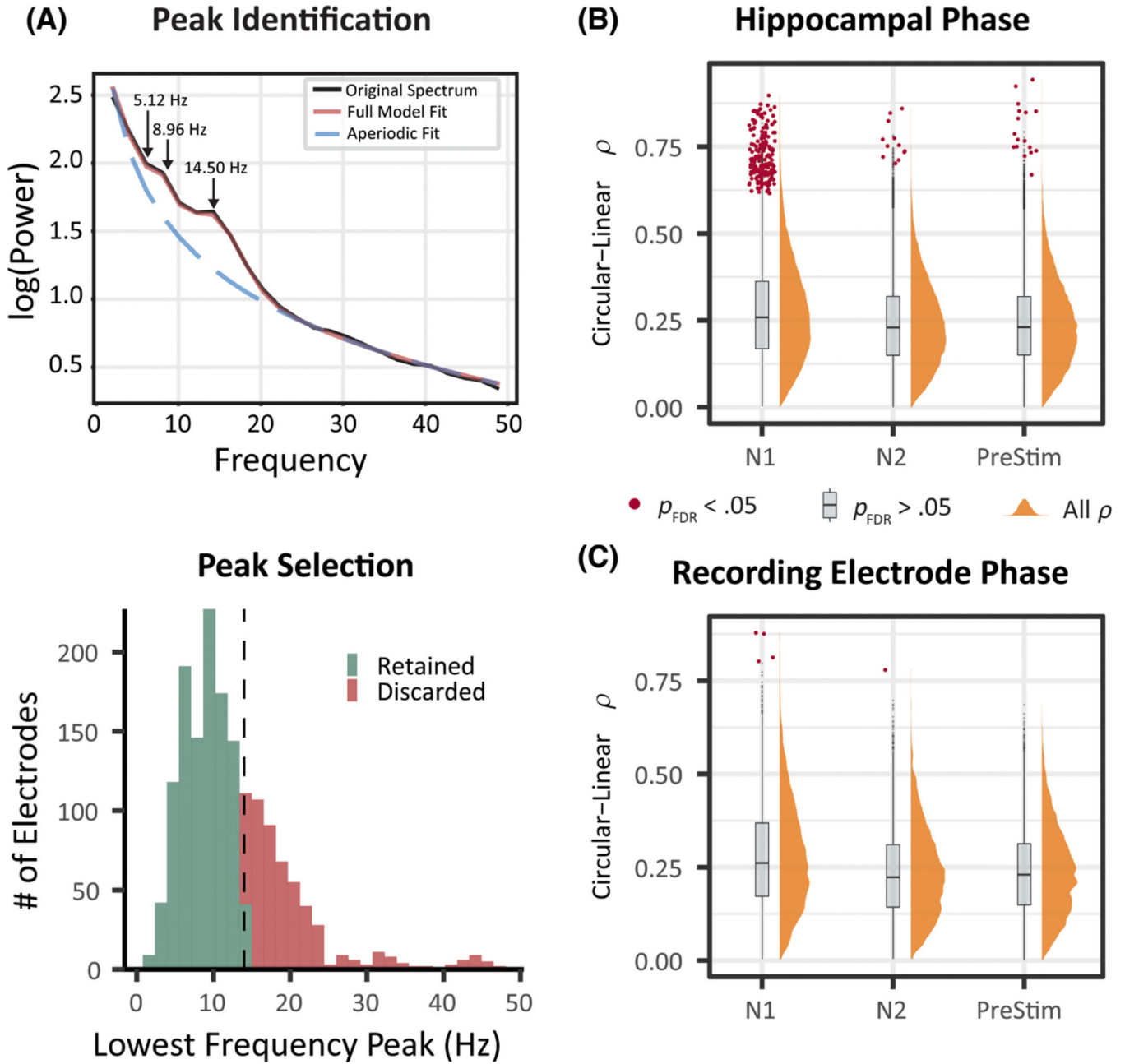
FIGURE 4.

Identification of low-frequency oscillations during stimulation and relationship with cortico-cortical evoked potential (CCEP) amplitudes. (A) Power spectrum (top subpanel) from one representative electrode with multiple low-frequency peaks. We selected the lowest frequency peak from each electrode with at least one peak in the 3–1 4 Hz range for further phase analysis (bottom subpanel). (B, C) For each CCEP, we computed the circular-linear correlation between either hippocampal phase (B) or recording electrode phase (C) and N1 amplitude, N2 amplitude, or amplitude during the −300 to −200 ms period relative to stimulation ("PreStim") as a negative control. Here, we plot the distributions of these circular-l inear correlations, stratified by pFDR < .05. All correlations are plotted in the

orange histogram, correlations with pFDR < .05 are plotted as red dots, and correlations with pFDR > .05 are shown as a gray boxplot.
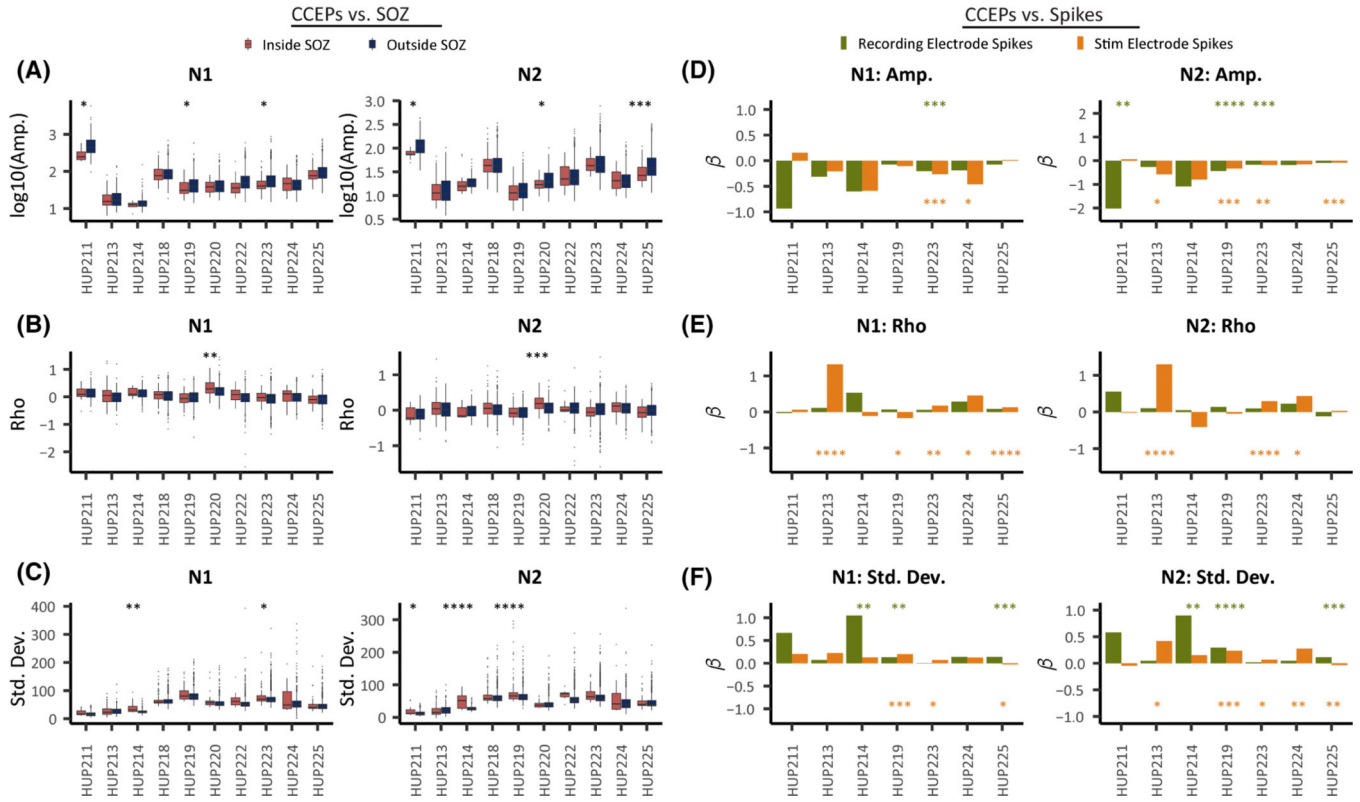
FIGURE 5.
Localization of cortico-cortical evoked potential (CCEP) measures relative to epileptogenic tissue. (A−C) Partial residuals (*y*-axis) of log-transformed CCEP amplitude (A), monotonic trend strength Spearman ρ (B), and standard deviation of CCEP amplitude across trials (C) with respect to a binary indicator of CCEP location inside (red) or outside (blue) the seizure-o nset zone (SOZ). Analysis was repeated for each patient (*x*-axis), and for N1 (left) and N2 (right). Asterisks represent the significance level from the regression coefficient for the SOZ indicator for each patient (see Section 2 for formulation). (s) Coefficients from linear regression model (β, *y*-axis) for log-transformed CCEP amplitude (D), monotonic trend strength Spearman ρ (E), and standard deviation of CCEP amplitude across trials (F) as a predictor of spike rate of recording (green) or stimulating electrodes (orange). Analysis was repeated for each patient (*x*-axis), and for N1 (left) and N2 (right). Asterisks represent significance level for regression coefficients. All *p*-values were adjusted to a false discovery rate (FDR) of $q < .05$ across all patients separately for each model formulation, that is, within each panel. *pFDR $< .05$. **pFDR $< .01$. ***pFDR $< .001$. ****pFDR $< 10^{-6}$.

TABLE 1

Patient characteristics.

HUP ID	Age (y)	Sex	Seizure-onset zone	AEDs during CCEPs	Home AEDs	Seizures with stim
HUP211	23	Male	Bilateral temporal	Same as Home Meds	Brivaracetam, cannabidiol, pregabalin	No
HUP212	55	Male	No seizures	Same as Home Meds	Clobazam, lamotrigine	No
HUP213	21	Male	Left SMA, mesial temporal	Same as Home Meds	Lacosamide, zonisamide	No
HUP214	40	Male	Bilateral mesial temporal	Same as Home Meds	Cenobamate, clobazam, lacosamide	No
HUP218	49	Female	Right mesial temporal	Same as Home Meds	Lacosamide, zonisamide, clonazepam	No
HUP219	26	Male	Right hippocampus	Same as Home Meds	Levetiracetam, lamotrigine	No
HUP220	49	Female	Left hippocampus	Same as Home Meds	Valproic acid, lacosamide, clobazam	No
HUP222	32	Female	Left temporal broad onset	Same as Home Meds	Brivaracetam, clobazam, topiramate	No
HUP223	46	Male	Left hippocampus	Reduced lacosamide	Lacosamide	No
HUP224	42	Female	Bilateral or right mesial temporal	Reduced zonisamide	Zonisamide	Yes, FAS (atypical)
HUP225	33	Male	Right anterior superior lateral temporal pole	Off home medications	Levetiracetam, lacosamide	Yes, FAS (typical)

Note: Demographic and clinical characteristics for all patients included in this study. HUP212 had no seizures or interictal discharges recorded on scalp EEG, but an epileptogenic focus was suspected based on MRI lesion (left mesial temporal sclerosis with chronic infarct of the left anterior insula and frontal operculum) and concordant semiology. Left mesial temporal interictal discharges were present on the intracranial evaluation. The focal aware seizure for HUP224 consisted of anxiety, odd sensation in throat, and coughing, which was atypical given that she typically only had impaired awareness seizures without preceding aura. CCEP data obtained during focal aware seizure for HUP224 was discarded. The focal aware seizure for HUP225 was typical semiology.

Abbreviations: AEDs, anti-epileptic drugs; CCEP, cortico-cortical evoked potential; FAS, focal aware seizure.