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Quantitative electrocorticographic biomarkers of clinical outcomes in mesial temporal lobe epileptic patients treated with the RNS® system

Sharanya Arcot Desai [a,*], Thomas K. Tcheng [a], Martha J. Morrell [a,b]

[a] NeuroPace, Inc., Mountain View, CA, USA
[b] Stanford University, Stanford, CA, USA

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HIGHLIGHTS

- Chronic ambulatory baseline ECoGs captured over 7 years from mesial temporal lobe (MTL) epilepsy patients were analyzed.
- Interictal spike rate strongly differentiates MTL patients with best clinical outcomes.
- Baseline ECoGs captured during months with no clinical seizures had the least interictal spike rate.

ABSTRACT

Objectives: Find interictal electrocorticographic (ECoG) biomarkers of clinical outcomes in mesiotemporal lobe (MTL) epilepsy patients.
Methods: In the NeuroPace® RNS® System clinical trials with 256 patients, 20 MTL patients with the most reduction in clinical seizures at Year 7 compared to baseline (upper response quartile; −96.5% median change) and 20 with the least reduction in clinical seizures (lower response quartile; −17.4% median change) were evaluated. Clinical and interictal ECoG features from the two response quartiles were compared.
Results: Demographic and clinical features were similar in the upper and lower response quartiles. Interictal spike rate (ISR) was substantially lower ($p < 0.0001$) in the upper quartile patients, while normalized theta (4–8 Hz) and normalized gamma (>25 Hz) were also different ($p < 0.05$) between the two response quartiles. ISR was positively correlated ($p < 0.05$) with clinical seizure rates in 71% of the channels analyzed. ECoG records captured during months with no clinical seizures had the lowest ISR.
Conclusions: ISR is a strong differentiator of clinical response in MTL patients. Normalized theta and gamma also differentiates clinical response.
Significance: In MTL patients, the interictal spike rate along with spectral power computed from chronic ambulatory baseline ECoGs may serve as biomarkers of clinical outcomes and maybe used as treatment endpoints.

1. Introduction

Epilepsy affects about 1% of the world's population. Current treatment options include antiepileptic medications (Kwan et al., 2011; Picot et al., 2008; Schiller and Najjar, 2008), surgical resection or ablation of the affected brain areas (Jobst, 2015) and electrical stimulation with FDA approved devices such as vagus nerve stimulation (VNS) (Schachter and Saper, 1998), deep brain stimulation of thalamic nuclei (DBS) (Salanova et al., 2015), and direct brain responsive stimulation of the region of seizure onset (the RNS® System) (Bergey et al., 2015; Morrell, 2011). Assessing the effectiveness of treatment is a major challenge in treating epilepsy. Patient-reported clinical seizures are often unreliable (Hoppe et al., 2007) since patients may be unaware of seizures because the seizures are subtle, because consciousness is impaired during or after a seizure (Blum et al., 1996; Blumenfeld, 2012), or because seizures occurred during sleep (Kerling et al., 2006).

Abbreviations: FDA, Food and Drug Administration; DBS, Deep Brain Stimulation; EcoG, Electrocortocogram; VNS, Vagus Nerve Stimulation; RNS, Responsive Neurostimulation.

* Corresponding author at: NeuroPace, Inc., 455 N. Bernardo Avenue, Mountain View, CA 94043, USA.
E-mail address: sdesai@neuropace.com (S. Arcot Desai).

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Objective epilepsy biomarkers could supplement patient-reported clinical seizures (Engel et al., 2013) and help to assess trends in the rate of clinical seizures. Identification of biomarkers could help to monitor changes in the underlying epilepsy over time, reduce the time it takes to identify effective antiepileptic drugs and doses (Skarpaas et al., 2018), and help to quickly identify effective stimulation and detection settings for neuromodulation devices (Engel, 2011).

Several potential biomarkers have been identified. High frequency oscillations (HFOs) are reported to be an excellent biomarker of epileptogenesis and have been used to delineate the epileptogenic zone in resection surgeries (Bragin et al., 2010; Engel et al., 2009; Fedele et al., 2017; Jacobs et al., 2010; Worrell et al., 2011). Imaging and molecular assessments have also been used as biomarkers of epileptogenicity (Pitkanen et al., 2016a, 2016b, 2011). Others have reported brain inflammation to be a biomarker for disease development (Vezzani et al., 2013). While these biomarkers have important prognostic value in epilepsy, the value of these metrics in the long-term assessment of treatment of persons with epilepsy is not defined.

Interictal spikes are associated with epilepsy, but conflicting relationships between spikes and seizures have been reported. Some studies have shown that the spike rate is unchanged or reduced prior to seizures, suggesting that spikes may play a protective role against seizures (Engel and Ackermann, 1980; Gotman and Marciani, 1985; Librizzi, 2003). Other studies have shown that spikes precede seizures (Avoli et al., 2006). In a study in 15 subjects with data collected with an intracranial seizure warning system over 2 years, changes in spike rates during the preictal period (1 h preceding seizures) compared to the interictal period (at least 8 h before any seizure and more than 8 h after a seizure) were variable; the spike rate significantly increased in 3 subjects, significantly decreased in 6 subjects and remained unchanged in the remaining 6 subjects (Karoly et al., 2016). Although these studies analyzed interictal spikes in the context of forecasting seizures minutes to several hours prior to seizure onsets, their value in predicting disease states or clinical outcomes have not been fully analyzed. A study of VNS in 19 patients with severe childhood epilepsies found significant positive correlations between the mean seizure frequency and the mean spike rate over 2 years in all patients, however, only 3 patients had a $\geq$50% reduction in seizures compared to baseline, so an analysis of the spike rate in responders vs non-responders could not be performed (Ebus et al., 2004). Furthermore, the analysis relied on a limited data set from 30-min EEG recordings performed once every 6 months over 2 years of treatment (Ebus et al., 2004).

The NeuroPace® RNS® System is a responsive brain stimulation device that is an adjunctive treatment for patients with medically intractable partial onset epilepsy having 1–2 seizure foci. The RNS System includes a cranially-implanted neurostimulator that is connected to up to 2 quadripolar cortical strip or depth leads, each containing 4 electrode contacts. The leads are placed at that patient's seizure focus or foci. The neurostimulator continuously monitors brain activity and when patient-specific abnormal patterns are detected, stimulation is automatically administered. In addition, the neurostimulator captures short recordings of electrographic activity. A feasibility and a randomized controlled pivotal trial of the RNS System included 256 patients with medically intractable partial onset seizures. Data collected included patient and epilepsy characteristics, and daily clinical seizure rates starting 3 months prior to implantation of the device and continuing every day over a median follow-up of 8.9 years. Data collected from the patients whose seizures arose from mesial temporal regions were analyzed to find electrographic biomarkers that could be relevant to clinical seizure frequency and thus helpful to assess the patient's clinical status.

2. Materials and methods

2.1. Terminology

In this paper, the term ECoG refers to long-term intracranial recordings captured using the RNS System and unless otherwise specified, the term ECoG record refers to one ECoG file containing up to 4 channels of ECoG data and the term ECoG channel refers to each channel of data in an ECoG record.

2.2. ECoG acquisition

Of the 256 patients in the RNS System clinical trials (Bergey et al., 2015; Morrell2011), 111 had seizure onsets in mesiotemporal lobe (MTL) regions and were included in this analysis (126 had neocortical onsets and 19 had both mesiotemporal and neocortical onsets). The types of ECoG records stored by the neurostimulator were specified by the physician as part of the patient's clinical care. Only ECoG records stored at specific times of day selected by the treating physician (i.e., "Scheduled ECoGs") were used in these analyses. Scheduled ECoG records serve as the patient's interictal baseline. The time of scheduled ECoG capture specified by the treating physician may vary from from patient to patient, and may also vary within patient over time. In total, ~150,000 of the 450,000 ECoG records were stored according to time(s) of day. Other types of ECoG records were stored because device detections exceeded a pre-specified duration criteria ("long episode" ECoG records), because amplifiers saturated, or because the patient swiped a magnet to indicate that a seizure had occurred. These records, which could include electrographic seizures, were excluded from these analyses. Note that electrographic seizures are not necessarily evident to the patient, therefore, for this analysis, clinical seizures are defined as patient reported seizures. Irrespective of the storage reason, ECoG records are typically 90 seconds long and typically contain 4 channels of differential ECoG data sampled from adjacent electrodes at 250 Hz per channel. Two leads, each with 4 electrodes, are connected to the neurostimulator and a single lead typically contributes 2 channels of data to an ECoG record.

2.3. Determining upper and lower clinical response quartile groups

All study protocols were approved by the institutional review boards of participating investigation sites. All participants gave written informed consent.

For the analyses, a month is defined as 28 days, a quarter is 84 days and a year is 336 days. Of the 111 MTL patients, 78 had at least 2 quarters of patient reported seizure counts available during Year 7 of treatment with the RNS System and were included in this analysis. Quarterly clinical responses were calculated as change in quarterly patient-reported clinical seizure counts since implant of the RNS System compared to a 3-month baseline period before the implantation of the RNS System. The mean clinical seizure responses in Year 7 were calculated as the mean of 2 to 4 quarterly clinical responses. Based on the mean clinical seizure response data in Year 7, the upper clinical response quartile group consists of the 20 MTL patients with the greatest reduction in seizures (i.e., highest clinical seizure response), and the lower clinical response quartile group consists of the 20 MTL patients with the lowest reduction in seizures (i.e., the lowest clinical seizure response).

The rationale for only selecting patients in the two extreme clinical outcome quartiles was to make the discovery of any electrographic seizure biomarkers relatively easy, since patient groups

with large differences in clinical outcomes may have large differences in electrographic features.

2.4. Assessing differences in demographic and clinical features between patients in the upper and lower clinical response quartiles

Fourteen demographic and clinical features were compared between the upper and lower quartile patients. Significance tests for each feature were carried out using the test which best matched the distribution of feature values being evaluated. Chi-Square or Fisher's Exact test was used for clinical/demographic features with binary values such as the presence or absence of an anatomical abnormality, while Wilcoxon Rank Sum or T-test (for normally distributed data) was used for features with non-binary distributions such as the age of epilepsy onset. SAS version 9.4 was used for computing statistical significance.

2.5. Comparison of ECoG features in patients in the upper and lower clinical response quartiles during Year 7 of treatment with the RNS System

Scheduled ECoG records saved during Year 7 of treatment were used for this analysis. Scheduled ECoG records with $\geq$10 s of continuous device detections were not included in any analyses reported in this paper in order to exclude scheduled ECoG records that captured electrographic seizures by chance. Further, a patient inclusion criterion of least 5 scheduled ECoG records captured during Year 7 was set. Previously published methods were used for extracting electrographic features, namely interictal spike rate (interictal spikes/sec), total spectral power, spectral power in delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–25 Hz), low gamma (20–50 Hz) and high gamma (>50 Hz) bands from each channel of the ECoG record (Skarpaas et al., 2018; Sun et al., 2018). In brief, interictal spikes were identified by first filtering data on each of the ECoG channels through a 10–100 Hz bandpass filter and then applying an amplitude threshold of 7.5 times the standard deviation of the filtered signal where the standard deviation was computed separately for data on each ECoG channel. Spike rates detected with this algorithm are very similar to those detected using a half-wave based algorithm (Sun et al., 2018). Welch periodograms (MATLAB function pwelch) were used for computing total spectral power and spectral power in each of the 6 classic frequency bands: delta, theta, alpha, beta, low gamma and high gamma. Normalized spectral power in each frequency band was computed by dividing the spectral power in each band by the total spectral power. For details on ECoG feature extraction methods, please refer to a previous publication by our group (Sun et al., 2018). Density 2D histogram plots or density scatter plots (example Fig. 1B and C) for visualizing ECoG features in each response quartile were created using MATLAB's hist3 function. The number of histogram bins in the density scatter plots was empirically chosen to produce reasonable data resolution. For comparing ECoG features from different groups through density scatter plots, it is necessary to match the number of ECoG features sampled from the groups being compared. This was done by sampling from the group with larger number of ECoG features to match the number of ECoG features available in the group with the smaller number of ECoG features using MATLAB's datasample function. For creating loglog density scatter plots (example Fig. 1C), +1 was added to the ECoG features to avoid encountering undefined values which can result when computing the logarithm of 0. For statistically comparing ECoG features from the upper and lower response quartile groups, median feature values were calculated for each ECoG channel and the two-tailed Wilcoxon rank sum test (MATLAB function ranksum) was used for computing statistical differences between the two groups. ECoG channels that were affected by surgical lead revisions or neurostimulator lead connection changes were excluded from the analysis.

PL/SQL Developer (Allround Automations) was used for making Oracle database queries and MATLAB 2017b was used ECoG feature extractions, correlation analysis, significance testing, and data visualization.

2.6. Assessing differences in ECoG features in patients from the upper and lower clinical response quartiles over all 7 years of treatment with the RNS System

Patients in the Year 7 upper and lower response quartile groups who had a minimum of 5 scheduled ECoG records captured during every year post implant (n = 8 patients in each quartile) were selected for this analysis. Electrographic features were extracted from data on all ECoG channels of scheduled ECoG records captured at every year since implant. Median feature values were computed for each ECoG channel at every year since implant i.e., one median value for total spectral power, spectral band power and interictal spike rate was computed for each ECoG channel at every year since implant. The two-tailed Wilcoxon rank sum method was used for computing statistical differences between the upper and lower quartile groups at each year since implant. Data for Year 1 was restricted to only contain ECoG records captured 6 months after the initial device implantation because transient changes in ECoG features following implantation of depth or of strip leads in the brain (Sun et al., 2018) can last for up to 5 months post implant.

2.7. Assessing temporal correlations between the interictal spike rate and seizure rate in patients in the upper clinical response quartile

Moving averages of clinical seizure rates and interictal spike rates (per ECoG channel) were computed for patients in the upper quartile group (n = 8 patients) who had at least 5 scheduled ECoG records collected at every year since implant. A window length of 3 months (84 days) with a step size of 1/4 the window length (21 days) was used for computing the moving averages. Spearman and Pearson correlations (MATLAB function corr) were used for computing correlations significance between clinical seizure rates and spike rate.

2.8. Assessing correlations between monthly clinical seizure rates and monthly interictal spike rates in all patients in the upper and lower clinical response quartiles

Moving averages of patient reported clinical seizure rates and average spike rates were computed using 1 month (28 days) windows with a step size of half the window length (14 days). Daily averages of spike rates were first computed for each ECoG channel in a patient when multiple scheduled ECoGs were stored on a given day and then moving averages were computed based on the daily averages. The inclusion criteria was a minimum of 3 days of ECoG data for any given 28-day window. Spearman and Pearson correlation coefficients were computed to assess rank and linear correlation relationships between monthly seizure rates and monthly spike rates in all patients in the upper and lower quartiles.

3. Results

3.1. Demographics and clinical information

The patients in the upper response quartile (n = 20) had a median change in the rate of clinical seizures of −96.5% with respect to baseline, while the patients in the lower response quartile (n = 20)

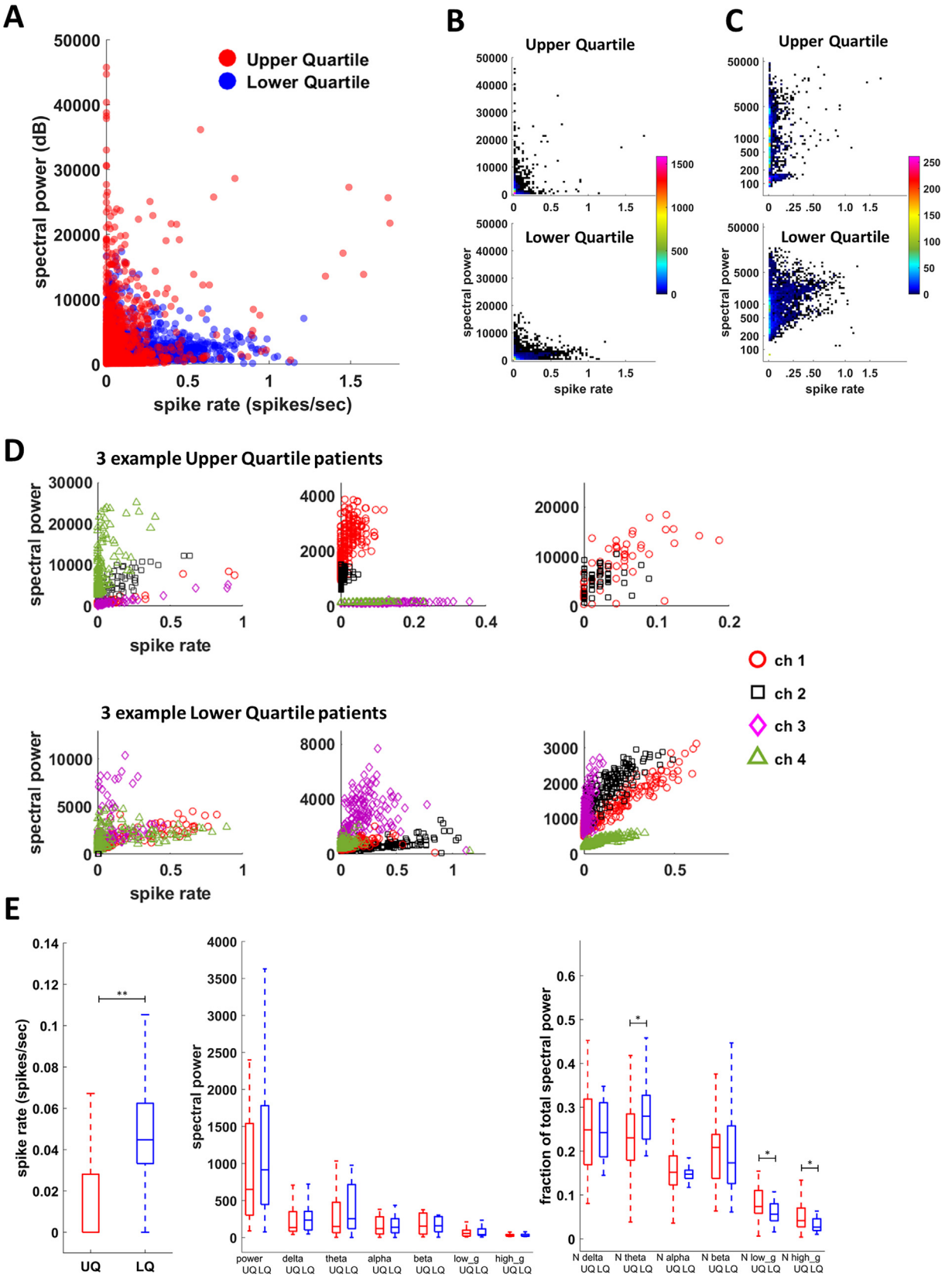
A
Upper Quartile
Lower Quartile
spectral power (dB)
spike rate (spikes/sec)
B
Upper Quartile
Lower Quartile
spectral power
spike rate
C
Upper Quartile
Lower Quartile
spectral power
spike rate
D
3 example Upper Quartile patients
spectral power
spike rate
3 example Lower Quartile patients
spectral power
spike rate
ch 1
ch 2
ch 3
ch 4
E
spike rate (spikes/sec)
UQ
LQ
spectral power
power
delta
theta
alpha
beta
low_g
high_g
fraction of total spectral power
N delta
N theta
N alpha
N beta
N low_g
N high_g

Table 1
Provides demographic and clinical information for the upper and lower clinical seizure response quartile groups.

Variable	Upper Quartile ($N = 20$) value (n) or mean ± std[†]	Lower Quartile ($N = 20$) value (n) or mean ± std[†]	P-Value	Test
Number of seizure onset regions	1 (9), 2 (11)	1(3), 2(17)	0.0824	Fisher's Exact
Number of AEDs at enrollment	2.45 ± 0.89	2.9 ± 0.72	0.1223	Wilcoxon Rank Sum Test
Age at enrollment	40 ± 12.71	35.75 ± 12.05	0.2848	T-test
Duration of epilepsy	17.2 ± 11.96	15.15 ± 10.86	0.5738	T-test
Gender	F(13), M(7)	F(11), M(9)	0.5186	Chi-square
Anatomic abnormality by brain imaging	Yes(11), No(9)	Yes(13), No(7)	0.5186	Fisher's Exact
Dysplasia	Yes(2), No(18)	Yes(1), No(19)	>0.999	Fisher's Exact
Encephalomalacia	No(20)	No(20)	n/a	
Sclerosis	Yes(9), No(11)	Yes(11), No(9)	0.5271	Chi-square
Tumor	No(20)	No(20)	n/a	
Vascular etiology	Yes(1), No(19)	No(20)	>0.999	Fisher's Exact
Subpial transection	No(20)	No(20)	n/a	
Resection	Yes(3), No(17)	No(20)	0.2308	Fisher's Exact
Prior intracranial monitoring	Yes(10), No(10)	Yes(7), No(13)	0.7515	Chi-square

[†] Whichever is applicable between binary value (Yes/No) or mean ± standard deviation is shown.

had a median change in the rate of clinical seizures of −17.4% with respect to baseline. Demographic and clinical features were analyzed to determine whether these could account for the differences in clinical outcomes. None of the 14 features, which included the number of seizure onset regions (1 or 2), number of antiepileptic drugs at enrollment, age at enrollment, duration of epilepsy, gender, presence of an anatomical abnormality on brain imaging, dysplasia, encephalomalacia, sclerosis, tumor, vascular etiology, subpial transection, resection or prior intracranial monitoring were different between the two groups at a p-value criterion of 0.05 (Table 1).

3.2. Electrographic differences between patients in the upper and lower response quartiles during Year 7

Thirteen patients in the upper response quartile and 10 patients in the lower response quartile had at least 5 scheduled ECoG records (range 9–738 in the upper quartile and 14–296 in the lower quartile) at Year 7 of treatment and were included in this analysis. The 13 patients in the upper quartile had a 97.9% median reduction in clinical seizures and the 10 patients in the lower quartile had a median reduction of 22.8%. Electrographic features extracted from scheduled ECoG records from the two response quartiles are shown in Fig. 1. Overall, interictal spike rate was lower in the upper quartile patients compared to the lower quartile patients, as shown in Fig. 1A, B and C, while the total spectral power appeared to have a similar distribution in the upper and lower quartile patient groups. Differences in the distribution of interictal spike rates between patients in the upper and lower response quartiles are seen most clearly on a loglog density scatter plot (Fig. 1C). Note that the linear density scatter plot (Fig. 1B) and the loglog density scatter plot (Fig. 1C) contain equal number of data points for the two response quartiles. As detailed in the methods section, this was achieved by randomly sampling from the upper response quartile group ($n = 7014$ data points from 13 patients) to match the number of data points ($n = 3622$ data points from 10 patients) from the lower response quartile group.

The distribution of the interictal spike rate and total spectral power across ECoG channels is also different within individual patients, as illustrated in Fig. 1D by examples from 3 patients in the upper quartile (top) and 3 from the lower quartile (bottom). For individual patients, within-channel variability is lower than between-channel variability. This supports summarizing each ECoG channel within each patient using a median value (i.e., each patient can have up to 4 median values, one for each ECoG channel), as was used in a study that analyzed ECoG changes over time in cortical strip and depth leads (Sun et al., 2018). The distributions of median values of interictal spike rate, spectral power in 7 frequency bands and normalized band power in 6 frequency bands for upper quartile ($n = 46$ ECoG channels from the 13 upper quartile patients) and lower quartile patients ($n = 36$ ECoG channels from the 10 lower quartile patients) are compared in Fig. 1E. The interictal spike rate was significantly lower in the upper quartile patients ($p < 0.00001$; two-tailed Wilcoxon rank sum test), but total spectral power and power in the 6 classic frequency bands were not different. Normalized power in the theta band was lower in upper quartile patients ($p = 0.01$), while normalized low gamma and high gamma were higher in upper quartile patients ($p < 0.05$). However, the statistical significance of the differences in the normalized spectral power bands between the two groups was modest compared to the interictal spike rate.

Fig. 1. (A) Scatter plot showing interictal spike rate (spikes/second) and spectral power (dB) in upper (red; n = 7014 data points from 13 patients) and lower (blue; n = 3622 data points from 10 patients) response quartile groups. Spike rate and spectral power are computed and plotted for each channel of each ECoG record captured during Year 7 of treatment, the same period over which the upper and lower quartile patient groups were defined. (B) Density scatter plots show spike rate and spectral power in the upper (top plot; n = 3622 sampled data points) and lower (bottom plot; n = 3622 data points) quartile groups. Density scatter plots consist of several 2D (spike rate and spectral power) histogram bins. Colormap shows the number of ECoG features in each 2D histogram bin. For example, bins with >1500 ECoG features are shown in bright pink and bins with very few (~1) ECoG features are shown in dark blue. (C) Density scatter plots with data for upper and lower response quartile groups plotted on loglog axes instead of linear axes as in 1B shows distribution of interictal spikes and spectral power in the two response quartiles more clearly than in 1B. (D) Scatter plot of spike rate and spectral power from 3 upper and 3 lower quartile patients showing relative within-channel consistency. Different colors (red, black, purple and green) represent data from different channels from a single patient. (E) Based on within-channel consistency, median feature values computed for each ECoG channel in a patient during Year 7 (up to 4 per patient; $n = 46$ ECoG channels from the 13 upper quartile patients and $n = 36$ ECoG channels from the 10 lower quartile patients), are used to create box and whisker plots showing the distribution of ECoG feature values for the upper (UQ; red) and lower quartile (LQ; blue) groups. X axis labels N delta - N high_g in the rightmost subplot refer to normalized power in delta to high gamma bands $^{**}p < 0.0001$; $^{*}p < 0.05$ with Wilcoxon Rank Sum test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Electrographic differences between patients in upper and lower clinical response quartiles from implant to Year 7

Eight patients each in the upper and lower response quartiles had at least 5 scheduled ECoG records captured at every year of treatment with the RNS System and were included in this analysis. In ECoG channels from upper clinical response quartile patients, there was an overall decrease in the spike rate and the clinical seizure rate over 7 years (median percent seizure reduction 100% at Year 7), while in ECoG channels from the lower clinical response quartile patients, neither the spike rates nor the clinical seizure rates changed substantially over time (median percent seizure reduction 22.8% at Year 7). This is illustrated in density scatter plots of spike rate vs total spectral power in ECoG channels in upper and lower clinical response quartile groups over Years 1 to 7 of treatment with the RNS System (Fig. 2A). A noticeable decrease in the spike rate over time in ECoG channels from the upper clinical response quartile group can also be seen in linear scatter plots (Supplementary Fig. 1) created with all available data points (i.e., not sampled as is the case with the density scatter plots) from the upper and lower clinical response quartile patients. Supplementary Fig. 2 provides examples of scatter plots of spike rates and spectral power over time in six example ECoG channels, 3 from upper quartile and 3 from lower quartile patient channels.

To verify that the overall decreasing trend in the spike rate is observed across the upper quartile group and is not driven by just a few ECoG channels, box and whisker plots computed on median spike rate and spectral power within each ECoG channel in the two groups of patients ($n = 26$ ECoG channels for upper quartile patients and $n = 30$ ECoG channels for the lower quartile patients) at every year of treatment are shown in Fig. 2B.

Beginning at year 3 of treatment with the RNS System, patients in the upper and lower quartiles had significant differences in spike rates, while the total spectral power remained similar in the two groups, as provided in Table 2. At Year 3, the spike rate was significantly lower ($p < 0.01$) in the patients in the upper quartile compared to the spike rate in the patients in the lower quartile and that difference between the two groups increased significantly over Years 3 to 7. At Year 7, normalized theta and normalized high gamma power were also significantly different between the patients in the upper and lower response quartiles, although these differences were modest when compared to the interictal spike rate differences.

3.4. Temporal correlations between spike rate and patient reported clinical seizure rates.

Changes in spike rates followed similar trends as changes in clinical seizure rates on several ECoG channels from the upper

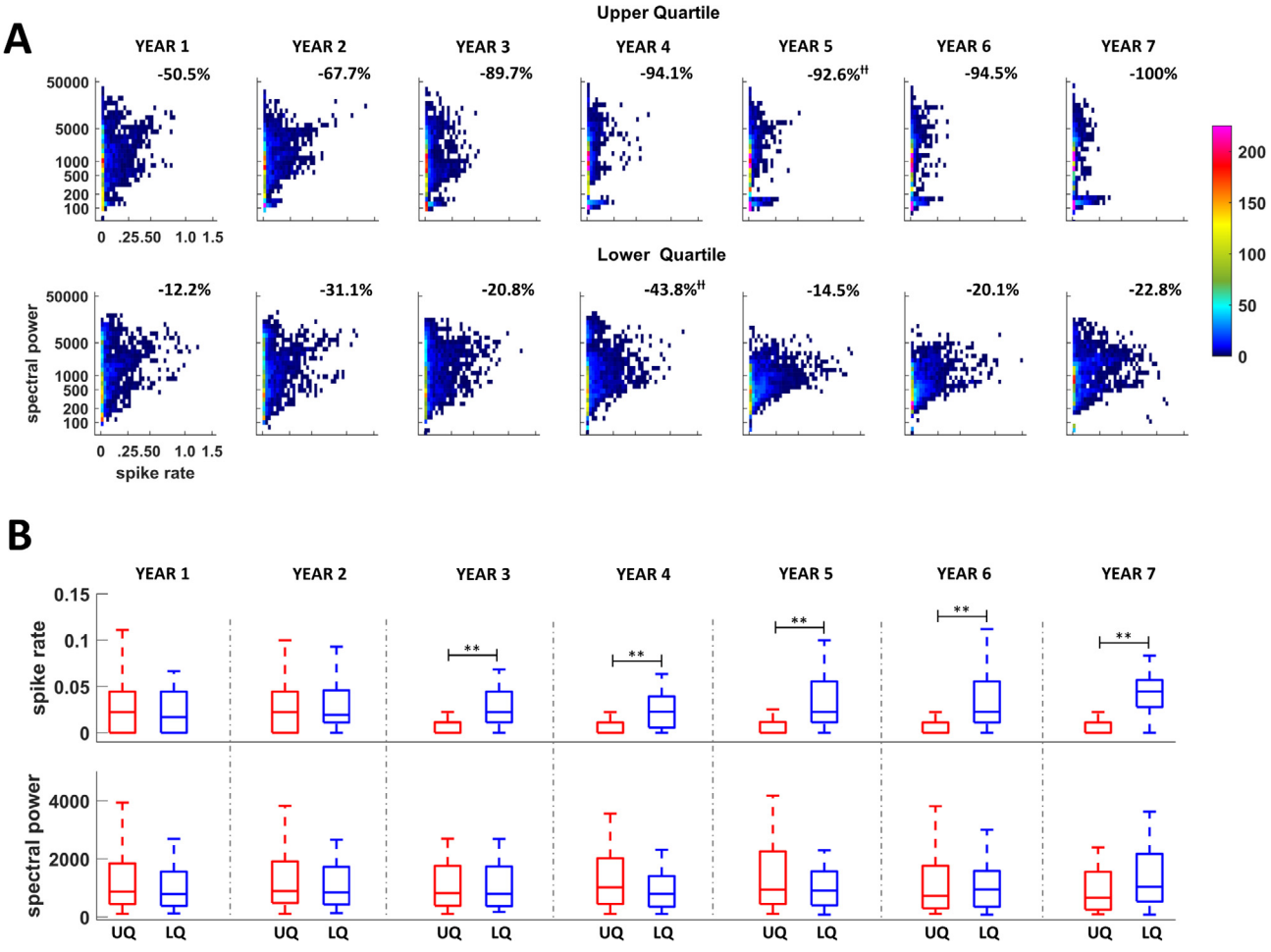

Fig. 2. (A) Density scatter plots of spike rate (spikes/second) vs total spectral power (dB) from ECoG channels in upper and lower quartile groups ($n = 8$ patients in each group) from Year 1 to Year 7 post-implant of the RNS System. Median change in clinical seizure rate for the upper and lower response quartile groups at every year of treatment compared to baseline is shown within each subplot. For example, −50.5% is the median change in clinical seizures for the upper quartile patients during Year 1 of treatment. Colormap shows number of ECoG features in each 2D histogram bin. ‡‡Clinical seizure rate data was available only for 7 out of 8 patients in this quartile during this year. (B) Box and whisker plots of spike rate and total spectral power computed with the median ECoG feature value for each ECoG channel ($n = 26$ ECoG channels from 8 upper quartile patients and $n = 30$ ECoG channels from 8 lower quartile patients) at every year since implant. $^{**}p < 0.01$ with Wilcoxon Rank Sum test.

Table 2
Statistical comparisons (*p*-values) between ECoG features in patients from the upper and lower response quartiles.

FEATURES	***YEAR 1***	***YEAR 2***	***YEAR 3***	***YEAR 4***	***YEAR 5***	***YEAR 6***	***YEAR 7***
SPIKE RATE	0.88067	0.69621	**0.00239***	**0.00074****	**0.00029****	**0.00007****	**0.00000****
SPECTRAL POWER	0.67526	0.50582	0.63963	0.43517	0.54874	0.95415	0.26043
DELTA	0.66330	0.44490	0.66330	0.66330	0.45476	0.94107	0.22723
THETA	0.98034	0.62792	0.94107	0.57085	0.49537	0.69944	0.18059
ALPHA	0.76118	0.55974	0.67526	0.47482	0.28189	0.90194	0.35329
BETA	0.72392	0.85015	0.95415	0.73627	0.69944	0.74869	0.57085
LOW GAMMA	0.98034	0.94107	0.95415	0.73627	0.65142	0.68731	0.78633
HIGH GAMMA	0.92800	0.77373	0.79900	0.57085	0.51638	0.52705	0.88894
N DELTA	0.72392	0.69944	0.91496	0.95415	0.63963	0.88894	0.63963
N THETA	0.59339	0.85015	0.43517	0.90194	0.85015	0.16507	**0.04592***
N ALPHA	0.91496	0.65142	0.71164	0.98034	0.59339	0.91496	0.66330
N BETA	0.52705	0.19717	0.46473	0.22723	0.35329	0.83729	0.52705
N LOW GAMMA	0.60480	0.86304	0.54874	0.52705	0.24677	0.43517	0.06223
N HIGH GAMMA	0.77373	0.76118	0.63963	0.72392	0.55974	0.34483	**0.01840***

Bold face shows statistically significant p-values.
** $p < 0.001$.
* $p < 0.05$.

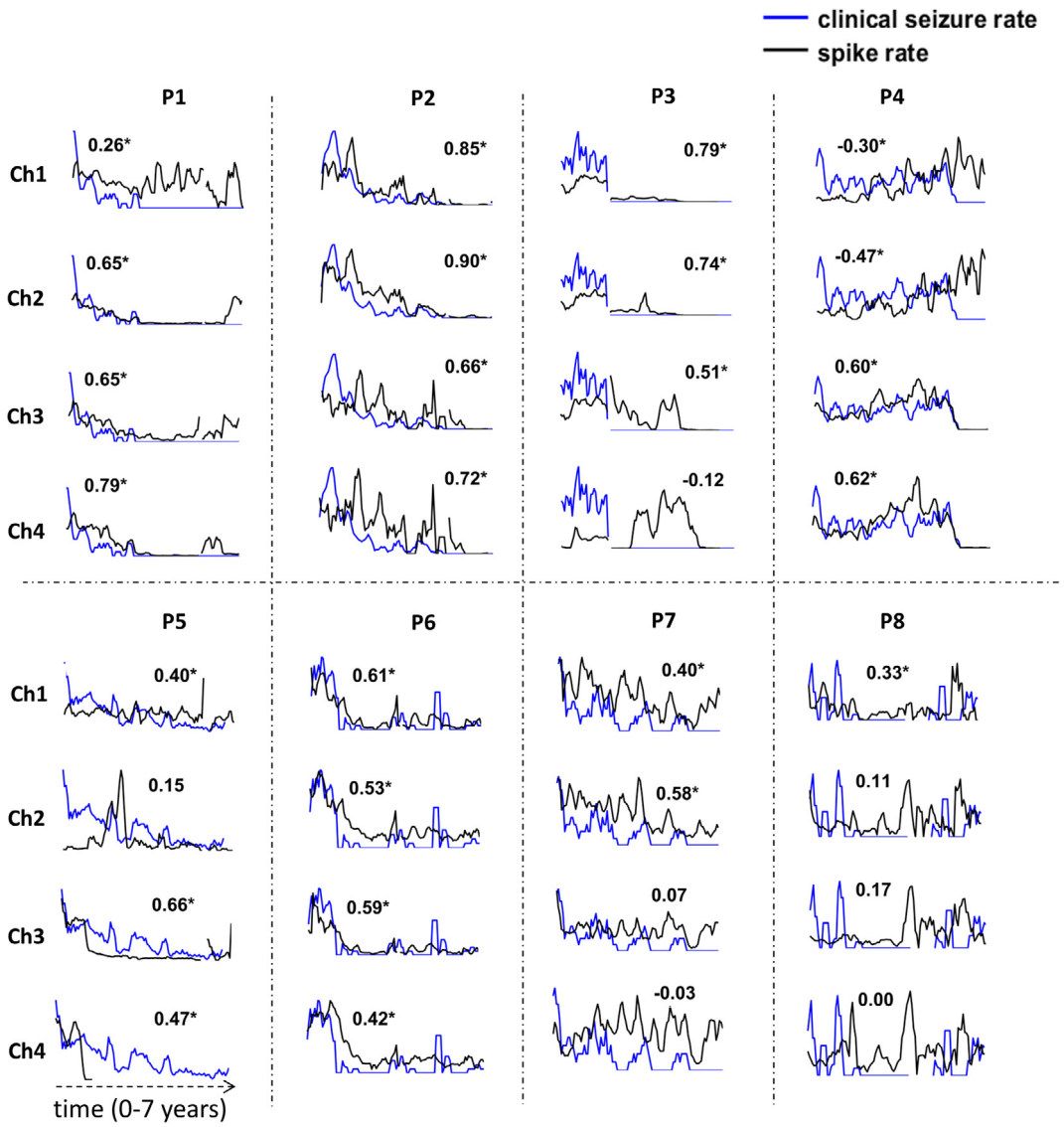

Fig. 3. Figure shows longitudinal plots of the average clinical seizure rate (seizures/day) (in blue) and the average spike rate (spikes/second) (in black) for each ECoG channel for all 8 upper response quartile MTL patients (P1-P8 are upper quartile patients 1 to 8 and Ch1-4 are channels 1 to 4). The Spearman correlation coefficients between the spike rate and clinical seizure rate are shown within each plot. An asterisk associated with the correlation coefficient indicates that the correlation coefficient is significant ($p < 0.05$).

Table 3
Summary of Spearman correlation computations between spike rates and clinical seizure rates from upper response quartile MTL patients. Table with Pearson correlation computations is included in the supplementary material (Supplementary Table 1).

Total number of channels analyzed	32	n/a
Channels with positive correlations	28	87.5%
Channels with significant positive correlations	23	71.2%
Channels with negative correlations	4	12.5%
Channels with significant negative correlations	2	6.3%
Total number of patients analyzed	8	n/a
Patients with 1 significant positive correlation channel	1	12.5%
Patients with 2 significant positive correlation channels	2	25%
Patients with 3 significant positive correlation channels	2	25%
Patients with 4 significant positive correlation channels	3	37.5%

response quartile patients. Mostly positive correlations between longitudinal trends in spike rates and patient reported clinical seizure rates can be seen in ECoG channels from the 8 upper quartile patients who had ECoG data captured at every year since implant (Fig. 3). With Spearman correlation computation (Table 3), three patients (1, 2 and 6) had significant positive correlations between the spike rate and the clinical seizure rate on all 4 channels while two patients (3 and 7) had significant positive correlations on three out of 4 channels. Patient 4 had 2 channels with significant positive correlations and 2 channels with significant negative correlations, while patient 7 had 2 channels with significant positive correlations and no correlation on the remaining 2 channels. Patient 8 had significant positive correlations between the spike rate and the clinical seizure rate on only 1 out of 4 channels, with the other three channels had no correlation. Overall, the Spearman correlation coefficients (which measures rank correlations between two variables) between spike rates and clinical seizure rates were significant and positive in 23/32 (71%), significant and negative in 2/32 (6%) and not significantly correlated in 7/32 (22%) ECoG channels. Every patient had at least one ECoG channel, and 7 out of 8 patients had at least 2 ECoG channels with significant positive correlations between the spike rate and the clinical seizure rate. Pearson correlation computations (which measures linear correlations between two variables) yielded similar results and are included in the supplementary material (Supplementary Table 1).

3.5. Correlations between the monthly spike rate and monthly clinical seizure rate in all upper and lower clinical response quartile MTL patients

Months with no clinical seizures have the lowest interictal spike rates. The relationship between the mean monthly clinical seizure rates (seizures/day) and mean monthly patient-channel interictal spike rates (spikes/second) from the upper and lower clinical response quartile patients are shown in Fig. 4 A and B. Both the Spearman and Pearson correlation coefficient between the monthly spike rate and monthly clinical seizure rate from all patients are positive (cc = 0.37 and 0.26 respectively) and significant ($p < 0.00001$). In Fig. 4B, the leftmost bin (i.e., first x-axis bin) contains average interictal spike rates for both upper and lower clinical response quartile patients recorded during months when the mean daily seizure rate was zero. The second bin contains interictal spike rates for clinical seizure rates of 0 to 0.25/day. The position of each bin to the right is moved by 0.25 seizures/day. The rightmost bin contains interictal spike rates for clinical seizure rates greater than 2 per day. A strong increasing trend in the interictal spike rate with increasing clinical seizure rates is seen over the 1st 4 bins, which have over 400 data points each. Variations in this trend are seen in the later bins presumably due to a smaller number of data points.

4. Discussion

4.1. Summary

Assessing the effectiveness of epilepsy treatments is a challenge in clinical practice. Relying on self-reports of seizures may not accurately reflect the extent to which that patient is burdened by epilepsy (Hoppe et al., 2007). Patients may not be able to recall their seizures, may be unaware of seizures unless they are witnessed, may not recognize seizures from sleep, or may forget or be unwilling to keep careful seizure diaries (Blum et al., 1996; Blumenfeld2012; Kerling et al., 2006). The clinician must decide whether to start, stop or change a treatment based on imperfect information that is available only every few months at the patient's clinic visit.

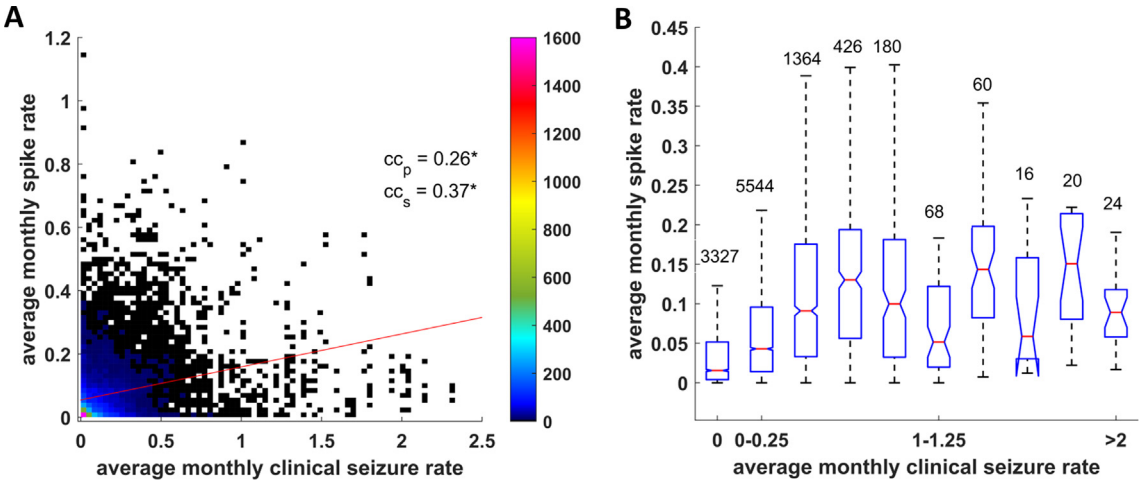

Fig. 4. (A) Density scatter plot of 7 years of average monthly ECoG channel interictal spike rates (spikes/second) (y-axis) and average monthly patient reported clinical seizure rates (seizures/day) (x-axis). The least squares fit line for data points from all upper and lower quartile patients shows a significant Spearman correlation coefficient of 0.37 (Pearson correlation coefficient is significant at 0.26). (B) Boxplot of monthly interictal spike rates (y-axis) binned by monthly clinical seizure rates (x-axis) in upper and lower quartile patients combined. The number of monthly ECoG channel data points is shown above each bin. For example, 3327 monthly interictal spike rate values (where one interictal spike rate value was computed per ECoG channel of an ECoG record) were computed from ECoG records captured during months with 0 clinical seizures. The median values for interictal spike rates in the bins from least clinical seizure rates to most seizure rates are 0.016 ($n = 3327$), 0.043 (5544), 0.091 (1364), 0.130 (426), 0.100 (180), 0.051 (68), 0.140 (60), 0.059 (16), 0.150 (20), 0.090 (24). Outliers are hidden.

Meaningful biomarkers indicating clinical improvement could support a more objective and quantitative approach to epilepsy treatment. The advent of chronically implanted brain recording devices brings the potential to discover electrophysiological biomarkers of the clinical response. The RNS System is a responsive direct brain neurostimulator, with leads implanted in the seizure focus/foci, that provides continuous electrocorticographic sensing, detects prespecified ECoG patterns, and records all detections as well as samples of selected types of ECoGs (Morrell2011). Electrographic seizures visible in ECoG recordings, as well as counts of prolonged detections of abnormal electrographic data ("long episodes") from the RNS System allow the user to estimate whether a patient's clinical seizure rate is increasing, decreasing or not changing. However, an alternative and potentially more sensitive biomarker may be available in electrographic features from non-seizure, baseline ECoG records. To address this question, the spike rate and spectral power of scheduled ECoG records were evaluated in patients with mesial temporal onset seizures who fell within the upper and lower clinical response quartiles of patients over 7 years of participation in RNS System clinical studies. This is the first study to analyze long term trends in baseline electrographic features in patients.

4.2. Clinical outcomes in upper and lower quartile patients at Year 7

At the end of 7 years of treatment with the RNS System, the median change in the clinical seizure rate compared to baseline was −96.5% for the 20 upper quartile patients, and −17.4% for the 20 lower quartile patients. Eight out of the twenty upper quartile patients were seizure free at Year 7 and all twenty upper response quartile patients had a >90% reduction in clinical seizure rate compared to baseline. To understand if demographic/clinical features could explain the substantial difference in clinical outcomes between the two groups, 14 clinical features were compared and none were significantly different. To find electrographic biomarkers of clinical outcomes, ECoG features from baseline ECoG recordings (i.e., scheduled ECoG records) were compared between the two groups. Findings from these analyses are summarized below.

4.3. Spike rate differentiates upper and lower response quartile patients at Year 7 of treatment

Thirteen patients who fell within the upper response quartile MTL patients had a significantly lower interictal spike rate ($p < 0.0001$) compared to 10 lower response quartile MTL patients at Year 7 of treatment, suggesting that hippocampal spikes are less frequent in clinically responsive patients. Indeed, 25/46 or 54.3% of the ECoG channels from the upper response quartile patients had a median spike rate of zero in their scheduled ECoG records, while very few of the ECoG channels in the lower response quartile patients had a median spike rate of zero (4/36 or 8.7% ECoG channels)

4.4. Spectral power differentiates upper and lower quartile patients at Year 7 of treatment

Spectral power differences were also observed between upper and lower response quartile patients at Year 7 of treatment. Normalized theta power was significantly less in the upper quartile patients and normalized low gamma and high gamma power was significantly higher in the upper quartile patients compared to the lower quartile patients ($p < 0.05$). A previous study on seizure prediction using the Freiburg EEG database (Park et al., 2011) analyzing data from 11 patients with seizure of neocortical onset, 8 patients with hippocampal onset seizures, and 2 patients with both hippocampal and neocortical seizure onsets found that spectral power features in the gamma range (>30 Hz) had the highest power for discriminating preictal and interictal states. Although the objective and design of the current study is different from the above-mentioned study on seizure prediction, it is interesting to note that spectral power in the gamma frequency range has been identified as a differentiating feature in both. However, in our study, the significance associated with spectral power features is modest compared to interictal spike rates, suggesting that for patients with seizures of mesial temporal onset, interictal spike rates are superior to spectral power in their ability to differentiate between good and modest responders to a treatment intervention. One potential explanation for this could be that abnormal EEG band power associated with brain abnormalities such as lesions (Fernandez-Bouzas et al., 1999) are present in both outcome quartiles. Responsive brain stimulation is unlikely to substantially change the underlying lesions and their associated spectral characteristics. Hence, a modest association between clinical outcomes and spectral power features may be observed.

4.5. Interictal spike rate changes over time, differentiating Year-7 upper and lower response quartile constant cohorts

Constant cohort analysis limited to patients having at least 5 ECoG records captured at each of 7 years of treatment showed that in the first two years, all electrographic features were similar in the upper and lower quartile patient groups. Statistical differences in the interictal spike rate started to emerge at Year 3 of treatment, which is also when the biggest year to year improvement in the clinical seizure rate for the upper response quartile patients was seen. Differences in electrographic features between the two response quartile groups continued to increase over time as sustained improvements in clinical seizure rates were observed in the upper quartile patients.

4.6. Correlations between interictal spike rate and clinical seizure rate over time

Spearman and Pearson correlation coefficients computed between clinical seizure rates and interictal spike rates over 7 years for the upper quartile patients were largely positive. There were positive correlations between the interictal spike rate and the clinical seizure rate in 88% of all channels (28/32) and these were significant for 71% (23/27). The interictal spike rate and the clinical seizure rate were negatively correlated in 4 channels (2 reached significance). All of the patients had significant positive correlations between the interictal spike rate and the clinical seizure rate in at least one channel, and 7/8 patients had significant positive correlations in at least 2 channels out of 4. One patient with bilateral mesial temporal seizure onsets had a particularly interesting trend; the 2 channels in the right hippocampal onset zone had significant negative correlations and the 2 channels in the left hippocampal onset zone had significant positive correlations. This may suggest that the negatively correlated interictal spikes are protective in one seizure onset zone in this patient, while the positively correlated spikes recorded from the contralateral seizure onset zone are proictal. In another patient with bilateral mesial temporal lobe seizure onsets, 3 channels (2 on one lead and one on the second contralateral lead) had significant positive correlations and the 4th channel had no correlation between the spike rate and clinical seizure rate. This could suggest that the distal two electrodes on the second lead are closer to the seizure onset zone than the proximal two electrodes. Thus, complex and inverse temporal relationships may exist between spike rates and clinical seizure rates. This relationship may depend on the location of leads with respect to the seizure onset zones, the anatomical location of

the seizure onset zones within the mesiotemporal lobes, the physiological role of spikes, for example, protective vs proictal, and the type of seizures and epilepsy, for example, focal aware vs generalized tonic clonic. These reasons may explain the conflicting reports on relationships between interictal spiking and seizure rates by several previous studies (Avoli et al., 2006; Engel and Ackermann, 1980). In our study, where unlike in the aforementioned reports, the objective was to analyze long term correlations between interictal spike rates and clinical outcomes, the number of channels with positive correlations between the interictal spike rates and clinical seizure rates largely outnumbered channels with negative correlations. This may be due to the fact that the patient population was largely homogenous i.e., all patients had focal epilepsies with mesial temporal lobe onsets.

4.7. Months with no seizures had the lowest spike rates

Months with low clinical seizure rates also had low interictal spike rates. When upper or lower clinical response quartile patients had no clinical seizures in any given month, the mean spike rate during that month was ~63% less (0.016 spikes/sec) than when the daily mean seizure rate was more than 0 but less than 0.25. Monthly spike rates trended upwards with increasing mean daily seizure rates. These findings strongly suggest that interictal spikes are positively correlated with clinical seizures in patients with MTL onset epilepsy and may be used as an objective measure of disease burden to supplement patient-reported clinical seizure rates

4.8. Clinical applications

One of the potential applications of these findings is to quickly and objectively assess effectiveness of new/modified treatments such as changes to neurostimulator device settings or medications. For example, patients who respond to certain antiepileptic medications have a decrease in the spike rate in the first month after the medication is started (Skarpaas et al., 2018). This information may be used by the treating physician as an objective outcome measure to inform decisions about continuing or changing the medication as early as one month after making the change. Another application could be to use the interictal spike rate as a measure to adapt stimulation delivered in a closed loop system where for example the amperage and/or frequency of stimulation is adjusted by a factor proportional to the baseline interictal spike rate. Indeed, ideas around using ECoG features to control stimulation parameters have been previously explored by several groups with some showing that adaptive stimulation is superior to continuous stimulation with fixed parameters for improving treatment outcomes in Parkinson's patients (Little et al., 2013; Rosa et al., 2015).

4.9. Limitations

Although strong positive correlations are seen between spike rates and seizure rates over 7 years of treatment with responsive stimulation to the seizure focus or foci, the causal relationship between interictal spikes and clinical seizure rates is not established. Continuous ECoG record storage, which the RNS System does not obtain, will likely be required to address this question.

Another limitation of the current study is that the analysis was limited to only patients with MTL onset seizures who fell into the upper and lower clinical response quartiles after 7 years of treatment with the RNS System. The large difference in clinical seizure rates between these groups made it relatively stratightforward to identify electrographic features that differentiate clinical outcomes. However, eliminating the middle two quartiles precludes correlation analyses between a wide range of clinical outcomes and electrographic seizure biomarkers which would increase the clinical relevance of these findings. Future analyses with data from all patients with MTL seizures, and from patients with seizures outside of the MTL, should be performed to support and complement these findings.

The focus of this study was to identify electrographic features that differentiate patients treated with the RNS System who fall into the upper and lower clinical response quartiles, irrespective of what caused changes in clinical seizure rates. Seizure rates were undoubtedly affected by ongoing epilepsy treatment including RNS System therapy and antiseizure medications, as well as other epilepsy therapies, health changes and life events. However, whatever the factors that contribute to clinical improvement are, there is strong evidence that interictal spikes are a biomarker of clinical response in MTL patients treated with the RNS System. It remains to be demonstrated whether interictal spikes are correlated with clinical outcomes in patients not treated with the RNS System. For example, changes in interictal spike rates in response to antiseizure medications could be measured with a long-term ambulatory EEG system to extend the findings of this study. Future analyses of large ambulatory ECoG data sets may identify additional biomarkers that correlate with clinical seizure rates. In this analysis, classic electrographic features such as spectral band power and interictal spikes were extracted from ECoG records. Additional features such as phase synchronization (Mormann et al., 2003) or cross frequency coherence, which have been shown to predict electrographic seizures, may have higher differentiating power than interictal spike rates and deserve study. Finally, machine learning methods with convolutional or recurrent neural networks may identify ECoG features which are superior to human-engineered features in differentiating treatment responders and nonresponders.

4.10 Conclusion

Retrospective analyses of long-term chronic ambulatory ECoG records from patients with seizures of mesial temporal onset who were treated with direct brain responsive stimulation with the RNS System indicate that the interictal spike rate is strongly and positively correlated with clinical seizure rates and is significantly different in epilepsy patients with seizures originating in the MTL who were in the upper and lower clinical response quartiles, as measured by a reduction in seizures with treatment. Normalized spectral power in the theta and gamma bands was also different between the two response quartiles. These interictal electrographic features may be used as biomarkers for clinical seizure frequency in patients with MTL epilepsy, and may serve as a rapid indicator of the likely effectiveness of specific epilepsy treatments.

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Declaration of Competing Interest

Authors Sharanya Arcot Desai, PhD, Thomas Tcheng, PhD and Martha Morrell, MD certify that they have equity ownership/stock options with NeuroPace and are employees of NeuroPace.

Supplementary material

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