Ripple oscillations in the left temporal neocortex are associated with impaired verbal episodic memory encoding

Zachary J. Waldman, Thomas Jefferson University
Liliana Camarillo-Rodriguez, Thomas Jefferson University
Inna Chervenova, Thomas Jefferson University
Brent Berry, Mayo Clinic
Shoichi Shimamoto, Thomas Jefferson University
Bahareh Elahian, Thomas Jefferson University
Michal Kucewicz, Mayo Clinic
Chaitanya Ganne, Thomas Jefferson University
Xiao-Song He, Thomas Jefferson University
Leon A. Davis, University of Pennsylvania

Only first 10 authors above; see publication for full author list.

Journal Title: Epilepsy and Behavior
Volume: Volume 88
Publisher: Elsevier | 2018-11-01, Pages 33-40
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1016/j.yebeh.2018.08.018
Permanent URL: https://pid.emory.edu/ark:/25593/v4cg6

Final published version: http://dx.doi.org/10.1016/j.yebeh.2018.08.018

Copyright information:
© 2018 Elsevier Inc.
This is an Open Access work distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Accessed May 30, 2020 10:20 AM EDT
Ripple oscillations in the left temporal neocortex are associated with impaired verbal episodic memory encoding

Zachary J. Waldman\textsuperscript{1}, Liliana Camarillo-Rodriguez\textsuperscript{1}, Inna Chervenova\textsuperscript{2}, Brent Berry\textsuperscript{6,7}, Shoichi Shimamoto\textsuperscript{1}, Bahareh Elahian\textsuperscript{1}, Michal Kucewicz\textsuperscript{6,7}, Chaitanya Ganne\textsuperscript{3}, Xiao-Song He\textsuperscript{3}, Leon A. Davis\textsuperscript{8}, Joel Stein\textsuperscript{9}, Sandhitsu Das\textsuperscript{10,11}, Richard Gorniak\textsuperscript{4}, Ashwini D. Sharan\textsuperscript{5}, Robert Gross\textsuperscript{13}, Cory S. Inman\textsuperscript{13}, Bradley C. Lega\textsuperscript{14}, Kareem Zaghloul\textsuperscript{15}, Barbara C. Jobst\textsuperscript{16}, Katheryn A. Davis\textsuperscript{12}, Paul Wanda\textsuperscript{8}, Mehraneh Khadjievand\textsuperscript{6,7}, Joseph Tracy\textsuperscript{3}, Daniel S. Rizzuto\textsuperscript{6}, Gregory Worrell\textsuperscript{6,7}, Michael Sperling\textsuperscript{3}, and Shennan A. Weiss\textsuperscript{1}

\textsuperscript{1}Dept. of Neurology and Neuroscience, Thomas Jefferson University, Philadelphia, PA USA 19107.
\textsuperscript{2}Dept. of Pharmacology & Experimental Therapeutics, Thomas Jefferson University, Philadelphia, PA USA 19107.
\textsuperscript{3}Dept. of Neurology, Thomas Jefferson University, Philadelphia, PA USA 19107.
\textsuperscript{4}Dept. of Radiology, Thomas Jefferson University, Philadelphia, PA USA 19107.
\textsuperscript{5}Dept. of Neurosurgery, Thomas Jefferson University, Philadelphia, PA USA 19107.
\textsuperscript{6}Dept. of Neurology, Mayo Systems Electrophysiology Laboratory (MSEL).
\textsuperscript{7}Dept. of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN USA 55905.
\textsuperscript{8}Dept. of Psychology, Mayo Clinic, Rochester, MN USA 55905.
\textsuperscript{9}Department of Radiology, Mayo Clinic, Rochester, MN USA 55905.
\textsuperscript{10}Penn Image Computing and Science Laboratory, Department of Radiology, Mayo Clinic, Rochester, MN USA 55905.
\textsuperscript{11}Penn Memory Center, Department of Neurology, Mayo Clinic, Rochester, MN USA 55905.
\textsuperscript{12}Dept. of Neurology, University of Pennsylvania, Philadelphia, PA USA 19104.

Corresponding Author: Dr. Shennan A. Weiss, 901 Walnut Street, PA 19107, Suite 400, Phone: (215) 503-7960, Shennan.Weiss@jefferson.edu.

Data and software availability
Analyzed data is available on a permanent Zenodo repository https://www.zenodo.org/record/838790#.WYStg9PyuWY, #2. Matlab, R, and SAS code used for data analysis is permanently available on Github https://github.com/shennanw/waldman_RAM/.

Conflicts of Interest
D.S.R. holds more than 5% equity interest in Nia Therapeutics, LLC (“Nia”) a brain stimulation device manufacturer. S.A.W. and Z.J.W. both hold more than 5% equity interest in Fastwave L.L.C., a EEG software manufacturer.

Ethical Publication Statement
The authors have read the journal’s position on issues involved in ethical publication and affirm their report is consistent with those guidelines.

Publisher’s Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Summary

Background: We sought to determine if ripple oscillations (80–120Hz), detected in intracranial EEG (iEEG) recordings of epilepsy patients, correlate with an enhancement or disruption of verbal episodic memory encoding.

Methods: We defined ripple and spike events in depth iEEG recordings during list learning in 107 patients with focal epilepsy. We used logistic regression models (LRMs) to investigate the relationship between the occurrence of ripple and spike events during word presentation and the odds of successful word recall following a distractor epoch, and included the seizure onset zone (SOZ) as a covariate in the LRMs.

Results: We detected events during 58,312 word presentation trials from 7,630 unique electrode sites. The probability of ripple on spike (RonS) events was increased in the seizure onset zone (SOZ, p<0.04). In the left temporal neocortex RonS events during word presentation corresponded with a decrease in the odds ratio (OR) of successful recall, however this effect only met significance in the SOZ (OR of word recall 0.71, 95% CI: 0.59–0.85, n=158 events, adaptive Hochberg p<0.01). Ripple on oscillation events (RonO) that occurred in the left temporal neocortex non-SOZ also correlated with decreased odds of successful recall (OR 0.52, 95% CI: 0.34–0.80, n=140, adaptive Hochberg, p<0.01). Spikes and RonS that occurred during word presentation in the left middle temporal gyrus during word presentation correlated with the most significant decrease in the odds of successful recall, irrespective of the location of the SOZ (adaptive Hochberg, p<0.01).

Conclusion: Ripples and spikes generated in left temporal neocortex are associated with impaired verbal episodic memory encoding. Although physiological and pathological ripple oscillations were not distinguished during cognitive tasks, our results show an association of undifferentiated ripples with impaired encoding. The effect was sometimes specific to regions outside the SOZ, suggesting that widespread effects of epilepsy outside the SOZ may contribute to cognitive impairment.

Keywords
verbal memory; epilepsy; high-frequency oscillation; epileptiform discharge

Introduction

Brief (1–50 msec) neurophysiological events such as action potentials in single neurons\(^1\) or high frequency oscillations (80–600 Hz)\(^2\) can serve as a marker of memory formation or cognition. Conversely, other brief neurophysiological events, such as interictal epileptiform spikes, can act as markers of cognitive disruption. Patients with absence seizures, which
consist of long trains of generalized spikes, experience inattention and a disruption of consciousness. Focal interictal spikes are not typically perceived and do not interfere with consciousness, but may interfere with cognition. For example, human studies of verbal episodic memory have reported correlations between interictal discharge rate increases during memory encoding and impaired word recall. It is unknown if other brief neurophysiological events, besides epileptiform spikes, can also serve as markers of disrupted cognition.

In experimental animals, ripple oscillations (80–200 Hz), when generated in area CA1 of the hippocampus during the sharp wave ripple complex (SpW-R), are known for mediating memory encoding, consolidation, and recall. In patients with epilepsy, ripple oscillations detected in intracranial EEG (iEEG) recordings from unaffected hippocampus during sleep can also correlate with successful memory consolidation. Furthermore, during wakefulness, ripple oscillations have been shown to mediate cognitive processing and memory throughout the brain due to fast network synchronization. However, ripple oscillations also occur more often in brain regions known to be epileptogenic, regardless of whether they occur superimposed on interictal spikes (RonS), or the ripples are superimposed on background EEG oscillations (RonO).

As ripples can both subserve memory and demarcate epileptogenic tissue, the overall cognitive significance of the biomarker for patients with epilepsy is unclear. Limited evidence suggests that ripple rates do not positively correlate with memory function, but rather memory performance negatively correlates with ripple rates measured outside the seizure onset zone. Thus, ripple events may sometimes promote or possibly disrupt memory.

In this study, we investigate a possible correlation between ripple and spike occurrences in macroelectrode iEEG recordings during word encoding trials of a list learning task, and changes in the probability of recall. No a priori assumptions were made regarding the pathological or physiological nature of each ripple event. We utilized a large cohort of medically refractory focal epilepsy patients who performed a list learning free recall task while undergoing iEEG evaluation for epilepsy surgery with depth electrodes. We focused on delineating the neuroanatomical regions susceptible to modulation by ripple and spikes to better understand the precise effect of ripples, and to help resolve the functional hubs of the network that mediate human verbal episodic memory.

Materials and methods

Subjects

Patients undergoing iEEG monitoring as part of the pre-surgical treatment for drug-resistant epilepsy were recruited in this multi-center study. Data were collected from: Thomas Jefferson University Hospital (Philadelphia, PA), Mayo Clinic (Rochester, MN), Hospital of the University of Pennsylvania (Philadelphia, PA), Dartmouth-Hitchcock Medical Center (Lebanon, NH), Emory University Hospital (Atlanta, GA), University of Texas Southwestern Medical Center (Dallas, TX), and Columbia University Medical Center (New York, NY). The research protocol was approved by each respective IRB and informed consent was obtained from all participants.
consent was obtained from each subject. Electrophysiological recordings were collected from clinical subdural and depth electrodes (AdTech Inc., PMT Inc.).

**Electrode localization and defining the location of the SOZ**

Coordinates of surface and depth electrode contacts were obtained for all subjects from post-implantation CT scans. Pre-implantation volumetric T1-weighted MRI scans were coregistered to the CT scans as well as to the MNI152 standard brain to enable comparison of recording sites in a common space across subjects. Anatomic locations of the recording sites were derived by converting MNI coordinates to Talairach coordinates and querying the Talairach daemon. The seizure onset zone (SOZ) was clinically defined by visual inspection of ictal iEEG by clinicians at each of the data collection sites.

**Memory task**

Subjects participated in list learning free recall memory tasks. In order to facilitate the comfort of the patient, the task was organized into blocks. During each block 12 randomly chosen words were displayed on a computer screen. Each word was displayed for 1600 msec, and the inter-word interval was jittered between 750–1000 msec. Lists were chosen from a pool of high-frequency nouns (available at [http://memory.psych.upenn.edu/WordPools](http://memory.psych.upenn.edu/WordPools)). Following the word display block the subjects performed an arithmetic distractor task for an average of 20 seconds. Participants were then provided with an average of 30 seconds to verbally recall the words in any order. Patients performed up to 25 blocks per session and some patients performed more than one session.

**Intracranial EEG data**

Intracranial data were recorded using either a Nihon Kohden EEG-1200, Natus XLTeK EMU 128 or Grass Aura-LTM64. The iEEG signals were sampled at either 500, 1000 or 1600 Hz and were referenced to a common contact placed either intracranially, on the scalp, or the mastoid process. A bipolar montage was calculated after recordings for each subject. All bipolar derivations with sampling frequency >500 Hz were low-pass filtered < 250 Hz and then down-sampled to 500 Hz. To examine the effects of ripples and spikes on encoding, we analyzed iEEG during both the 750 msec of inter-word-interval, and 1600 msec during word display. We also analyzed iEEG during the entirety of the distractor and recall tasks for each block.

**Detecting candidate ripple events in iEEG recordings**

Prior to data preprocessing we first determined which depth electrode iEEG recordings exhibited a signal to noise ratio sufficient for accurate ripple detection (Supplementary Methods).

Following the exclusion of recordings from electrode contacts with excessive high frequency artifacts we selected the time intervals of the recordings that would be subject to our analysis. During encoding, the jitter between each word trial varied between 750–1000 msec. Due to this variability we selected for our analysis of the word encoding trial iEEG recordings which concatenated the final 750 msec of pre-word interval with the 1600 msec
Classifying and characterizing ripple events in iEEG trials using topographical analysis

To determine whether the candidate ripple event was a true ripple or a result of filter ringing from a sharply contoured spike or artifact, we applied a topographical analysis to the time-frequency (TF) plot. To develop an automatic software method for classifying ripples as true or false, we utilized the difference in the TF representation of sharp transients and true high frequency oscillations. TF plots of time series data exhibit an inherent topography defined by isopower contours. A true ripple is represented by a “blob” of power within the ripple band (80-250 Hz), and if contour lines are defined for a TF representation of the “blob”, with the maximum and minimum frequencies confined to the ripple band, the contours will have closed loops. In contrast, a false ripple is represented by a “candle” of power in the ripple band (Figure 1D), but importantly this “candle” continues below the ripple band. Thus, when the contour lines are defined for the “candle” within the ripple band, the contours will have open loops (Supplementary Methods). This same method was used to derive the mean spectral content, duration, and power of each ripple event (Supplementary Methods). Since some of the EEG amplifiers used in this study had an anti-aliasing filter at 125 Hz, we removed all the ripple events with a mean frequency greater than 120 Hz from the dataset.

Identification of interictal discharges in the iEEG using a topographical analysis of timefrequency plots

Both true and false ripple events were subsequently processed by a second stage algorithm designed to identify the presence of an interictal discharge, within 200 ms of the ripple event, on the basis of an analysis of TF plots resulting from wavelet convolution. This algorithm operated under the principle that interictal discharges produce a recognizable
motif in the TF plot that is relatively independent of both the amplitude and slope of the iEEG during the discharge (Supplementary Methods).

**Repeated measures logistic regression models (LRM) for word encoding trials**

As predictors in the statistical models, the absence of a ripple was coded ‘0’, ripple on spike (RonS) was coded ‘1’, spike was coded ‘2’, and ripple on oscillation (RonO) was coded ‘3’. The events recorded from individual electrodes were aggregated across the corresponding regions of interest. In the case of multiple events occurring within a single region, codes of higher number took precedence. If a given subject did not have coverage of a neuroanatomical region, then the data from that subject was missing for the region. The events of each word recall and proportions of word recall events in 12-word blocks were analyzed using the generalized linear mixed model (GLMM) model with the assumption of binomial distribution. The generalized estimating equation (GEE) estimation approach for population-average GLMM was used with the exchangeable working correlation matrix to account for correlations between rates of recall in repeated words within blocks, repeated blocks within experiment, and repeated experiments within the same subject. The GLMM model allows for variable number of observations per subject representing variable number of experiments and word blocks per patient. Separate models were fitted for each neuroanatomical region of interest. For each region, predictors of individual word recall included, the type of event and location of the corresponding electrode inside or outside the seizure-onset zone (SOZ), or whether the recording was made using a XLTEK EMU128 amplifier with a 131 Hz anti-aliasing filter (Natus, Pleasonton, CA). Predictors of proportions of word recall events in 12-word blocks included the type of events and the mean rates of RonS, Spike, and RonO per minute in the left temporal neocortex during the encoding, distractor and recall periods. If no events of interest occurred during the encoding or distractor or recall periods, then the corresponding predictors were equal to zero. The p-values from each set of models (including models for multiple regions of interest) were corrected for multiple comparisons using the adaptive Hochberg algorithm\(^\text{22}\). The data were analyzed in R (Vienna, Austria) and SAS 9.4 (Cary, NC).

**Block design repeated measures logistic regression models**

The GEE estimation approach for population-average GLMM was used with the exchangeable working correlation matrix to account for correlations between rates of recall in repeated blocks within the same subject. The predictors of word recall included the mean rates of RonS, Spike, and RonO per minute in the left temporal neocortex during the encoding, distractor and recall periods. If no events of interest occurred during one of these periods, then the corresponding predictors were equal to zero. Separate models were fitted using the mean rates for RonS, Spike, and RonO, because of linear dependency. The rates of word recall events in 12-word blocks were analyzed using the logistic regression model with assumption of the binomial distribution. The data analysis was performed in SAS (Cary, NC) and R (Vienna, Austria). Multiple testing correction was performed using the adaptive Hochberg algorithm in SAS\(^\text{22}\).
Results

Across 58,312 word trials of the free recall task (Figure 1A) the estimated overall word recall probability by session was 25.4 ± 11.6% (s.e.m). We applied a detector that implemented a wavelet topographical algorithm\textsuperscript{20,23} to detect and quantify ripple and spike events in depth iEEG recordings from 7,630 unique locations (Figure 1B) in 107 patients (Supplemental Table 1). The algorithm specified whether an iEEG trial beginning 750 msec prior to word presentation and ending at word display offset contained a) neither a ripple nor spike, b) a ripple on spike (RonS), c) a sharply contoured epileptiform spike, d) a ripple not on a spike \textit{i.e.} a ripple on background oscillation (RonO)(Figure 1C,D). The detector could not quantify multiple events per trial. The detector identified only ripple events over a predetermined power threshold corresponding to an amplitude threshold of >8 μV. We selected a relatively high threshold to maximize the specificity of the detector for ripple events likely to be annotated by a clinical epileptologist\textsuperscript{15}. The detector was applied to iEEG recordings from all the experimental trials across all patients and at every non-excluded recording electrode site. We then calculated the sensitivity, precision, and inter-rater reliability as measured by the intraclass correlation coefficient of the detector for the different event types in mesial temporal and neocortical sites in 448 electrodes from 8 patients (Supplementary Methods, Supplemental Table 2). Since the human validation of the detector was not blinded, but rather performed by adding and deleting marked events, the moderately high performance of the detector (sensitivity ~70–80%, specificity ~70–80%, Supplementary Table 2) was inflated.

We examined the probability and rate of ripple and spike events by electrode (Figure 1C), and by the lobe in which they occurred. The event rates computed by lobe were preprocessed for use in the logistic regression models (LRMs, see methods). Thus, these rates do not precisely correspond with event rates present in the continuous iEEG recordings in each electrode recording site (Supplementary Table 3). Overall, ripple events occurred with the greatest rate and probability in temporal and limbic (\textit{i.e.} mesial temporal including the cingulate gyrus) regions bilaterally (Supplemental Table 3). At the single electrode level, this was also the case (Figure 1C). Since the bilateral temporal and limbic regions had superior electrode coverage and mostly higher rates of spike and ripple events, we focused our study on these regions to limit errors from limited spatial and temporal sampling due to infrequent events.

To determine whether ripples and spikes in the bilateral temporal and limbic regions during encoding modulate the probability of recall, we constructed four repeated measures logistic regression models (LRMs). Since prior work has demonstrated that spikes occurring outside the seizure onset zone (SOZ) correlate with disrupted memory encoding, while spikes within the SOZ do not\textsuperscript{24}, we included the SOZ as a covariate in the LRM\textsc{s}. In our study the occurrence of RonS events was increased in the SOZ (SOZ: 0.3% of trials, non-SOZ: 0.09%, t=2.13, d.f.=81, p=0.036, paired t-test), but this was not the case for spikes or RonO events. Using the LRM\textsc{s} we first asked if spikes or ripples within the SOZ correlate with disrupted encoding (Figure 2, Supplementary Table 4). The LRM for ripple and spike events in the left temporal neocortex revealed that RonS events within the SOZ correlate with disrupted encoding (odds ratio [OR] of word recall 0.71, 95% confidence interval (CI): 0.59–0.85,
n=158). This effect remained significant after correction for multiple testing ($p_{\text{raw}} = 0.0001$, $p_{\text{adj}} = 0.001$, adaptive Hochberg method, n=24 tests).

We next utilized the four LRM to ask if ripple and spike events in the non-SOZ correlate with disrupted encoding (Figure 3, Supplementary Table 4). Using the LRM for the left temporal neocortex we found that RonO events in the non-SOZ correlate with disrupted encoding (OR 0.55, 95% CI: 0.39–0.77, n=140 events, $p_{\text{adj}}=0.003$). We also observed, using the LRM for ripple and spike events in the right limbic regions, that spikes in the right limbic non-SOZ correlate with disrupted encoding (OR 0.74, 95% CI: 0.60–0.92, n=524 events, $p_{\text{adj}}=0.036$).

Since ripples significantly correlated with disrupted encoding in the left temporal neocortex, and the left temporal neocortex is likely to be involved in verbal episodic memory encoding, we next asked whether ripples and spikes correlate with disrupted encoding in specific sub-regions of the left temporal neocortex (Figure 4, Supplementary Table 5). We constructed LRM of the superior, middle, and inferior left temporal gyrus, but did not use the SOZ as a covariate in these LRM because of sample size limitations due to electrode coverage. Using the middle temporal gyrus model, we found that RonS (OR 0.53, 95% CI 0.34–0.82, n=125, $p_{\text{adj}}=0.023$, adaptive Hochberg method, n=9 tests), and spikes (OR 0.61, 95% CI 0.53–0.34, n=743, $p_{\text{adj}}<0.001$) correlate with disrupted encoding. Using the inferior temporal gyrus model, we found that spikes also correlate with disrupted encoding (OR 0.51, 95% CI 0.33–0.78, n=106, $p_{\text{adj}}=0.01$). We also observed that RonO significantly correlated with disrupted encoding in the superior temporal gyrus (OR 0.60, 95% CI 0.44–0.83, n=33, $p_{\text{adj}}=0.009$, $p_{\text{adj}}=0.009$).

Since we observed that ripples with a mean frequency < 120 Hz recorded from the left temporal neocortex correlate with disrupted encoding, we next asked if including ripple events with a higher frequency content would influence the effect, and if a 131 Hz anti-aliasing filter utilized for a portion of the recordings, showed any interaction with the effect. We found a trend showing that RonS and RonO events that occurred during encoding in the left temporal neocortex, irrespective of the location of the SOZ, corresponded with a decreased probability of recall ($p_{\text{adj}}<0.07$). We also found no significant interaction between the use of an anti-aliasing filter and the effect of RonS and RonO events on recall probability (Supplementary Table 6).

We next sought to determine whether ripple and spike events that occur in the left temporal neocortex decrease the probability of recall only when they occur during the encoding epoch. We utilized a distinct LRM to correlate the mean rate of ripple events or spikes that occurred during either the encoding, distractor, or recall epochs in the left temporal neocortex with the number of items recalled per word encoding block (Figure 5, Supplemental Table 7). A distinct LRM was required because the distractor and recall epochs were not structured into trials. We could not use the SOZ as a covariate in this LRM because of sample size limitations due to the number of patients (N=42). We found that increases in the mean rate of RonS events in the left temporal neocortex during encoding, but not during the distractor, or recall, resulted in a decrease in the odds of recall (CI=0.92, 95% CI 0.9–0.95, $p_{\text{adj}}<0.001$, step up Bonferroni method, n=18 tests). The same correlation
was also seen for RonO events (OR=0.79, 95% CI 0.71–0.89, \( p_{\text{adj}} < 0.001 \), step up Bonferroni method, \( n=18 \) tests) (Figure 5, Supplementary Table 6.). In the case of spikes, a trend was evident during the encoding epoch (OR=0.94 95% CI 0.89–1.0, \( p_{\text{adj}} = 0.137 \)), and a correlation was evident during the distractor epoch, but the effect size was small (OR=0.96, 95% CI 0.95–0.97, \( p_{\text{adj}} < 0.001 \)).

**Discussion**

We demonstrate that during a list learning free recall task, when ripple events occur in the left temporal neocortex during word presentation the probability of successful recall is decreased. While other work has shown ripples promote memory consolidation\(^1^1\), the criteria for distinguishing the ripples that promote memory from those that correlate with disrupted memory encoding are not yet clear. The neuroanatomical location wherein the ripple occurs appears to be important, as does the location of the seizure onset zone. Paradoxically, left temporal neocortex ripples on spikes (RonS) only correlated with disrupted encoding when they occurred at the site of seizure generation, whereas left temporal ripples on background EEG (RonO) only correlated with disrupted encoding when they occurred outside the site of seizure generation. Furthermore, in the left temporal neocortex, ripples that occurred during the distractor and recall epochs appeared to have no effect on memory performance.

Our results suggest that, in patients with focal epilepsy, ripple events may serve both as a marker of memory processing\(^2\) and as a marker of memory interference. We did not explicitly distinguish the putative pathological ripples from other physiological higher-frequency oscillations in this study\(^1^5\). Since we sought to identify only those events that would be acceptable for annotation during visual inspection of the iEEG by a clinical epileptologist, we utilized an automated ripple detector that identified only relatively high amplitude events\(^2^0\). Both visual and automated ripple annotation have demonstrated that ripple rates are elevated in the SOZ during sleep and under anesthesia\(^1^5\). We also found that, during behavior, RonS event probabilities were elevated in the SOZ. However, it is unlikely that our detector defined events that were exclusively pathological, since large amplitude ripple events detected in hippocampal iEEG recordings have also been found to positively correlate with memory consolidation performance\(^1^1\). One difference between the ripple events defined in this study and the other studies examining the role of high-frequency activity in cognition\(^1^2\)–\(^1^4\), is that the events defined in this study occurred 2- to 3-fold less often due to the relatively high amplitude threshold of our detector.

In the left temporal neocortex, only RonS in the SOZ and RonO events outside the SOZ were associated with encoding disruption. However, the block design LRM (Figure 5), demonstrated that RonS and RonO events in the left temporal neocortex are associated with encoding disruption, irrespective of the location of the SOZ. Ung et al.\(^2^4\) recently reported that spikes outside the SOZ in the left hemisphere during encoding resulted in a decrease in the probability of recall. Ung et al. theorized that this observation supports the concept of a ‘nocifero us cortex’\(^2^4\) in which pathological activity interrupts normal electrophysiology and functioning in a region extending far outside of the primary epileptogenic zone. While Ung et al., did not measure ripple oscillations, our results support the ‘nocifero us cortex’ concept,
and highlight that activity associated with RonO events, correlate with disrupted cognition in the nociferous cortical territory. The mechanism by which ripple oscillations associate with a disruption of cognition is not yet established. A single prior study reported elevated ripple rates outside the SOZ in patients with impaired memory, while for patients with intact memory, this was not the case\textsuperscript{17}. An important avenue of future investigation is to determine if post-operative verbal episodic memory encoding deficits correlate with pre-operative ripples and spikes in the resected or unresected left temporal neocortex.

We also found that ripples and spikes in the left middle temporal gyrus (MTG) correlated with the most significant decrease in probability of successful recall, irrespective of the location of the SOZ. The left MTG is activated by tasks that probe semantic processing and memory\textsuperscript{25}, and lesion-mapping studies have shown that it is essential for word-level comprehension and retrieval\textsuperscript{26}. Perhaps the left MTG can be considered the most critical hub in the network of active and coordinated brain regions that mediate verbal episodic memory or semantic processing. Recently it was demonstrated that electrical stimulation of the left MTG during verbal episodic memory encoding can enhance the probability of recall\textsuperscript{27–29}.

The results of the LRMs did not demonstrate a significant correlation between ripples or spikes in mesial temporal structures and failed encoding, except in the case of spikes in the right mesial temporal and cingulate non-SOZ. However, the effect size in this region was relatively small. While some past studies have shown that spikes in the hippocampus correlate with disrupted encoding\textsuperscript{4–5}, other studies failed to replicate these findings\textsuperscript{6}. One possible explanation for this discrepancy is that if spatial granularity is too coarse in a study that utilizes pathologic events to map functional memory networks, the anatomy of that functional network may not be accurately characterized. In our study, we grouped all mesial temporal structures as well as the cingulate gyrus together in one category. We did not have the sample size required to examine the effects in entorhinal cortex and sub-regions of the hippocampus. Ripples and spikes in discrete mesial temporal regions and sub-regions may have diverging effects. Future investigations that utilize a larger number of patients, and accurate segmentation of mesial temporal structures prior to electrode localization, could hopefully resolve this controversy by demonstrating the effect of pathologic events in key structures in the mesial-temporal lobe.

The design of our study had several other shortcomings. In an effort to determine whether a ripple or spike would correlate with disrupted encoding, we deliberately analyzed the 750 msec preceding the word presentation, in addition to the word presentation. We included the iEEG recordings prior to the presentation because neuronal action potentials can be suppressed for 0.5–2 seconds following an epileptiform discharge\textsuperscript{30,31}. We did not relate events in the iEEG which occur during the 750 msec following word presentation to the recall of that word, despite the fact that encoding of that word may still be taking place. Another shortcoming was that we identified ripple and spike events in the entire encoding epoch, and did not explicitly examine the differential effects of events occurring at different stages or segments. For example, it is unclear if events in the occipital lobe would disrupt encoding only if the event occurs prior to or during the initial stages of word presentation\textsuperscript{32}. In addition, our design made it impossible to quantify the impact of multiple ripples or
spikes that occur during a single trial, and the cognitive effects of the wave component of spike-wave discharges\textsuperscript{33}.

Another major shortcoming of our study was that the iEEG sampling rate was only 500 Hz thereby reducing the probability of detecting and quantifying ripples with a frequency greater than 120 Hz. We found that among the ripples detected and quantified in this study most of the events had frequencies < 120 Hz. This finding is in accord with a large number of studies which quantified the properties of ripples measured using macroelectrodes in humans at sampling rates of 2000 Hz\textsuperscript{12,16,20}. The limited sampling rate of this study eliminated the possibility of detecting and quantifying fast ripples. Also, fast ripples may have been mistakenly identified as ripples due to aliasing. Fast ripples selectively disrupt single neuron reactivation during SpW-R in the rodent hippocampus\textsuperscript{34}, and could also disrupt human episodic verbal memory and spatial memory.

In summary, both epileptiform spikes and ripples correlate with a failure of verbal episodic memory encoding in the left temporal neocortex and may contribute to cognitive disorders in temporal lobe epilepsy\textsuperscript{35}. Future studies should be directed at understanding the mechanistic differences that distinguish pathological ripples from physiological ripples\textsuperscript{36}, and high-frequency activity\textsuperscript{13,14,27}. One strategy may be to examine differences in single unit activity during the high-frequency activity and ripple events that coincide with successful and failed verbal memory encoding.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgements**

We thank Blackrock Microsystems for providing neural recording and stimulation equipment. This work was supported by the DARPA Restoring Active Memory (RAM) program (Cooperative Agreement N66001–14–2–4032). The views, opinions, and/or findings contained in this material are those of the authors and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government. Dr. Weiss is supported by 1K23NS094633–01A1.

**REFERENCES**


Highlights:

1. Ripples that occur superimposed on epileptiform spikes correlate with disrupted verbal episodic memory encoding when they occur in the left temporal neocortex seizure onset zone.

2. Ripples that occur superimposed on background EEG correlate with decreased verbal episodic memory encoding when they occur in the left temporal neocortex non-seizure onset zone.

3. Irrespective of the seizure onset zone, the neuroanatomical structure wherein spikes and ripples on spikes most significantly correlate with disrupted encoding is the left middle temporal gyrus.
Figure 1: Mapping ripple and spike events in a cohort of 107 medically refractory epilepsy patients performing a verbal episodic memory task.

A. Illustration of the verbal episodic memory task including the encoding, distractor, and recall epochs. B. Illustrative brain rendering demonstrating the number of electrode contacts in each lobe across the entire patient cohort. C. Superimposition of the location of the intracranial depth EEG recording contacts in the cohort of 107 patients in a glass brain. The color of each electrode indicates the probability of a word presentation trial in which a ripple on spike (top), ripple on oscillation (middle), or spike (bottom) occurs in the iEEG. This
event probability is proportionate to event rate irrespective of the task. D. Example time-frequency spectrograms and corresponding iEEG traces for each of the event types. Panel D1 illustrates a distinct “blob”, representing the ripple, with a spectral content greater than a simultaneous “candle”, representing the spike. Panel D2 illustrates a distinct “blob”, representing the ripple. Panel D3 illustrates a distinct “candle” representing the spike. The distinction between the “blob” in D2 and “candle” in D3 is indicated by respective white arrows.
Figure 2: Ripples on spikes in the left temporal neocortex seizure onset zone during word encoding decrease the odds of successful word recall.

Bar graph of the odds ratio and 95% confidence interval of successful word recall given the occurrence of ripple and spike events in the seizure onset zone during verbal encoding. When ripple and spike events occurred in the seizure onset zone during encoding, only ripple on spike events in the left temporal neocortex resulted in a significant decrease in the odds of word recall after a correction for multiple testing (adaptive Hochberg method, **p<0.01, n=24 tests, N=number of patients, 10–50 blocks of 12 words per patient).
Figure 3: Ripples on oscillations in the left temporal neocortex non-seizure onset zone during word encoding decrease the odds of successful word recall.

Bar graph of the odds ratio and 95% confidence interval of successful word recall given the occurrence of ripple and spike events in the non-seizure onset zone during verbal encoding. When ripple and spike events occurred in the non-seizure onset zone during encoding, ripple on oscillation events in the left temporal neocortex, and spikes in the right mesial-temporal and cingulate regions resulted in a significant decrease in the odds of word recall after correcting for multiple testing (adaptive Hochberg method, **p<0.01, *p<0.05, n=24 tests, N=number of patients, 10–50 blocks of 12 words per patient).
Figure 4: Spikes in the left middle temporal gyrus during encoding correlate most significantly with a decrease in the probability of correct recall. Bar graph of the odds ratio and 95% confidence interval of successful word recall given the occurrence of ripple and spike events during verbal encoding, irrespective of the seizure onset zone. When ripple and spike events occurred in the left temporal neocortex during encoding, ripple and spike events in the left middle temporal gyrus, and spikes in the left inferior temporal gyrus resulted in a significant decrease in the odds of word recall after correcting for multiple comparisons (adaptive Hochberg method, **p<0.01, *p<0.05, n=6 tests, N=number of patients, 10–50 blocks of 12 words per patient).
Figure 5: Ripple events significantly reduce the odds of correct word recall when the events occur during the word encoding epoch, but not the distractor or recall epochs.

Bar graph of the change in the odds ratio of word recall predicted by an increase in the rate of ripple and spike events in the left temporal neocortex during the encoding, distractor, and recall epochs irrespective of the location of the seizure-onset zone adjusted for multiple testing (adaptive Hochberg method, *p<0.001, n=18 tests, N=42, 10–50 blocks of 12 words per patient). Odds ratio shown correspond to one event per minute increase in rates.