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Extrahippocampal high-frequency oscillations during epileptogenesis

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Summary

The current study aimed to investigate the spatial and temporal patterns of high-frequency oscillations (HFOs) in the intra-/extrahippocampal areas during epileptogenesis. Local field potentials were bilaterally recorded from hippocampus (CA1), thalamus, motor cortex, and prefrontal cortex in 13 rats before and after intrahippocampal kainic acid (KA) lesions. HFOs in the ripple (100–200 Hz) and fast ripple (250–500 Hz) ranges were detected and their rates were computed during different time periods (1–5 weeks) after KA-induced status epilepticus (SE). Recurrent spontaneous seizures were observed in 7 rats after SE, and the other 6 rats did not develop epilepsy. During the latent period, the rate of hippocampal HFOs increased at the ipsilateral site of the KA lesion in both groups, and the HFO rate was significantly higher in the animals that later developed epilepsy. Animals that later developed epilepsy also demonstrated widespread appearance of HFOs, in both the ripple and the fast ripple range, whereas animals that did not develop epilepsy only exhibited changes in the ipsilateral intrahippocampal HFO rate. This study demonstrates an association between an increased rate of widespread HFOs and the later development of epilepsy, suggesting the formation of large-scale distributed pathological networks during epileptogenesis.

Keywords

epileptogenesis; extrahippocampal; high-frequency oscillation; latent period; PIN clusters

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DISCLOSURE

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

1 | INTRODUCTION

During the past 2 decades, enormous contributions have been made to identify the role of high-frequency oscillations (HFOs) in relation to epileptogenesis and seizure genesis.^{1–3} In clinical epileptology, the identification of a close spatial association between the location of HFOs and the seizure onset zone has led to the integration of measurements of HFOs in presurgical examinations, as removal of regions with high rates of HFO occurrence is associated with a good postoperative outcome.² However, at present, the role of HFOs in relation to epileptogenesis is unclear. If a predictive role of HFOs is identified, it could have a significant benefit for the development of approaches to prevent and cure epilepsy.^{1,3} Prior studies in the intrahippocampal kainic acid (KA) rat model of temporal lobe epilepsy (TLE) revealed the occurrence of pathological HFOs (pHFOs) in the dentate gyrus ipsilateral to the lesions in animals that subsequently developed spontaneous seizures, whereas pHFOs were not found in those animals that did not develop epilepsy.⁴ Precise studies, such as assessment of voltage depth profiles, multiunit activity, and spike clustering, suggest that the appearance of pHFOs reflects summation of action potentials of synchronously bursting neurons, comprising discrete local net-works of pathologically interconnected neurons (PIN clusters),^{4,5} which play a critical role in epileptogenesis. These studies, however, focused on hippocampal or parahippocampal areas, such as subiculum, CA1, and dentate gyrus.^{4,6}

It has become evident that epilepsy is a network phenomenon and even focal epilepsy is associated with abnormal changes in connectivity between multiple brain areas.⁷ Therefore, research on HFOs needs to be extended to extrahippocampal structures. If the process of epileptogenesis requires formation of a widespread net-work of PIN clusters,⁵ pHFOs during epileptogenesis should also appear in areas outside of hippocampus (HP). In the present study, we carried out microelectrode recordings of multiple brain areas in an animal model of TLE, to investigate the changes in spatial and temporal patterns of HFO occurrence from baseline through the latent period of epilepsy in animals that did, and did not develop epilepsy.

2 | MATERIALS AND METHODS

2.1 | Animal preparation and data acquisition

Adult Wistar rats (250–300 g, $n = 17$) were anesthetized with 2.0% isoflurane during microelectrode implantation. A guide cannula was implanted above the CA3 (anteroposterior [AP] = -3.0 , mediolateral [ML] = 3.0 , dorsoventral [DV] = 2.5) region in the left posterior HP for KA injection. Fixed-recording microelectrodes consisting of a 50- μm diameter tungsten wire were then bilaterally implanted into the (1) prefrontal cortex (PC; left PC [LPC]/right PC [RPC]; AP = 3.7 , ML = ± 0.5 , DV = 3.0), (2) motor cortex (MC; left MC/right MC [RMC]; AP = 0.2 , ML = ± 4.0 , DV = 5.5), (3) thalamus (TH; left TH/right TH [RTH]; AP = -3.6 , ML = ± 2.6 , DV = 6.0), and (4) HP (left HP [LHP]/right HP [RHP]; AP = -6.0 , ML = ± 4.6 , DV = 4.5).⁸ Additionally, ground and reference electrodes were positioned above the cerebellum. All the procedures were approved by the University of California, Los Angeles Institutional Animal Care and Use Committee.

Local field potentials (LFPs) with a wide bandwidth range from 0.1 to 3.0 kHz (16 channels) were recorded using the Intan RHD 2000 digital system (Intan Technologies, Los Angeles, CA, USA). Beginning 1 week after microelectrode implantation, freely moving rats were plugged into the data acquisition system and signals were recorded for 7 days as the baseline condition. Then, $10.0 \mu\text{mol L}^{-1}/0.4 \mu\text{L}$ of KA was injected into the CA3 area of LHP. The animals were placed in their home cages and observed with 24-h/d video-electroencephalographic (EEG) monitoring for up to 5 weeks. Status epilepticus (SE) and chronic seizures were identified by reviewing animals' behavior and by offline review of raw EEG data based on the criteria suggested by Bragin et al.⁶ Animals were divided into 2 groups: those that developed spontaneous seizures (E+) and those that did not develop spontaneous seizures (E-).

2.2 | Data analysis

We separated our experiments into 6 recording periods (baseline and latent period [week 1-week 5]) for further data processing. Three to 10 minutes of LFPs during slow wave sleep were selected from each animal in each week by excluding motion artifacts and sleep spindles. The selected data were later imported to the EEGLAB⁹ for preprocessing. HFOs (100–500 Hz) were detected based on the method suggested by Staba et al.¹⁰ and performed in a MATLAB (MathWorks, Natick, MA, USA) toolbox RIP- PLELAB¹¹ (see Appendix S1 for details). The HFO data that were within the same group were combined into larger files, and the HFO events were detected based on onset of the oscillations. The rate of HFO occurrence (number per minute) was analyzed at each recording site during both baseline and latent periods. Wavelet analysis was then conducted to describe the time-frequency profile of the HFO events, which were further classified into ripples (80–250 Hz) and fast ripples (FRs; 250–500 Hz).⁴ Statistical analysis was performed using 1-way analysis of variance (ANOVA) for the comparison of total HFO rate in both E- and E+ groups. A Dunnett post hoc test was applied for multicomparison between baseline and latent periods (specifically, baseline vs week 1, week 2, week 3, week 4, and week 5 within each animal group). Two-sample t test with unequal variances was applied to compare the total HFO rate between E+ and E- groups in each recorded period.

3 | RESULTS

Four of 17 animals died either during KA treatment or during the first 3 weeks after SE. Electrographic recordings were successfully conducted in the remaining 13 animals. Among these animals, 7 developed epilepsy (E+) and 6 did not (E-). Types of seizure onsets and their patterns were similar to what was described in our previous publications^{12,13}; the quantification of seizure duration, occurrence, and type are described in Appendix S1.

3.1 | Spatial and temporal distribution of total HFOs during epileptogenesis

Quantitative analysis of total HFO rate (including both ripples and FRs, n/min) is shown in Figure 1. There was a statistically significant difference of total HFO rate between baseline and latent period in both the E- and E+ groups as determined by 1-way ANOVA (E-: $F_{5, 30} = 4.58$, $P = .003$; E+: $F_{5, 36} = 5.10$, $P < .001$). Specifically, a Dunnett post hoc test revealed that in the E- group, significant increases in total HFO rate were observed in week 1 (1.78

+ 1.29, $P=.02$), week 2 (1.54 ± 1.20 , $P=.03$), and week 4 (1.62 ± 0.45 , $P=.002$) compared to the baseline (0.28 ± 0.39). In the E+ group, increases in HFO rate occurred in all 5 latent periods (week 1: 2.08 ± 1.42 , $P=.03$; week 2: 3.20 ± 2.18 , $P=.008$; week 3: 3.92 ± 2.31 , $P=.002$; week 4: 4.11 ± 1.30 , $P<.001$; week 5: 2.11 ± 1.51 , $P=.034$) and were significantly higher than the baseline (0.62 ± 0.97).

Two-sample t test (unequal variances) revealed no significant differences in total HFO rates between E- and E+ groups at baseline (0.28 ± 0.39 vs 0.62 ± 0.97 , $P=.64$) and week 1 (1.78 ± 1.29 vs 2.08 ± 1.42 , $P=.68$). However, the differences in total HFO rate began to be noticeably higher in the E+ group from the second week after KA injection. From week 2 to week 4, the total HFO rates in the E+ group were +108% (3.20 ± 2.18 vs 1.54 ± 1.20 , $P=.03$), +833% (3.92 ± 2.31 vs 0.42 ± 0.29 , $P<.001$), and +154% (4.11 ± 1.30 vs 1.62 ± 0.45 , $P=.01$) higher than in the E- group. We did not observe a significant difference in HFO rates between the E+ and E- groups in week 5 at the $P<.05$ level, but the E+ group was significant higher at the $P<.1$ level (2.11 ± 1.51 vs 0.77 ± 0.18 , $P=.076$).

It was noticed that changes in the total HFO rates in the E- group during the latent period were primarily due to changes in HFO rate in ipsilateral HP (LHP). Specifically, the ratio of (LHP rate) / (total HFO rate) in the E- group was 70% in week 1, 90% in week 2, 50% in week 3, 77% in week 4, and 44% in week 5, whereas in the E+ group, these rates were 22%, 33%, 42%, 35%, and 7%, respectively.

3.2 | Ripple and FR occurrence at baseline and during the latent period

Ripple and FR events were observed throughout the entire recorded latent period in the animals that later developed epilepsy. Figure 2A–D shows examples of ripple and FR activity located in LHP (Figure 2A), TH (Figure 2B), MC (Figure 2C), and PC (Figure 2D) in the E+ group. Raw data, bandpass-filtered (100–500 Hz) data, and time-frequency profiles shown in these cases reveal both ripples and FRs during a 300-millisecond epoch.

To demonstrate the progression of HFOs during the latent period, we selected 10-minute HFO EEG sequences for each week in the E- (Figure 2E) and E+ groups (Figure 2F) and identified ripples (blue) and FRs (red). Starting at week 1, there was an obvious increase in ripples in LHP in both groups. However, only rats that later developed epilepsy (Figure 2E) revealed an increase of ripple events in other remote recording areas, such as LPC/RPC (week 1–5), TH (week 1–5), and contralateral HP (week 2–4). No obvious changes in ripple activity outside the ipsilateral HP were observed in the E- group, except for a small number of ripples noticed in ipsilateral PC (week 3 and week 5) and bilateral MC (week 1; Figure 2F). FRs were not observed in either group during the baseline period, but began to appear in the first week after KA injection. During week 1, FRs were only detected in LHP near the lesion site in the E- group (week 1; Figure 2E), whereas they occurred in LPC, RMC, RTH, and bilateral HP (LHP and RHP) in the E+ group (week 1; Figure 2F). FRs were not observed in the E- group for the rest of the latent period. Detailed measures of ripple and FR rates are shown in Table S1.

4 | DISCUSSION

This study investigated spatial and temporal features of HFOs in the intrahippocampal KA-induced SE rat model of chronic epilepsy during baseline and the latent period in rats that did and did not develop epilepsy. Our major finding is the existence of extrahippocampal HFOs during epileptogenesis after local lesion of HP. During the latent period, increases of total HFOs were observed in both groups, but total HFO rates in E+ animals were much higher than in E− animals. Widespread appearance of both ripples and FRs was only observed in the E+ group. FRs were only observed in the E− group at the site of KA injection in the first week, and the increase in ripple activity in the E− group occurred primarily within the lesioned HP. Overall, our results indicate a unique relationship between increased widespread ripple and FR HFOs and the later development of epilepsy, suggesting development of a large-scale—rather than local—pathological network during epileptogenesis.

Increased HFO rates after SE have been widely observed in experimental models of TLE, in HP, dentate gyrus, and entorhinal cortex.^{6,14–16} Bragin et al suggested that HFOs in both the ripple and FR ranges can be pathological after SE.⁶ Our observations of increased total HFOs in week 1 in both groups of animals could result from a nonspecific neuronal reaction after SE. In the animals that later developed epilepsy, the hippocampal HFO rate remained greater than the baseline over the entire latent period, which is consistent with our previous data.^{6,14} Our previous studies did not reveal any FRs in lesioned HP in animals that did not develop epilepsy.⁶ We speculate that the few FRs observed in week 1 in the E− group reported here represent transient pathological neuron firing due to the acute lesion effect. It must be considered, however, that some rats that did not develop epilepsy within the time frame of this study might have developed epilepsy later if they had been allowed to survive.

A unique finding of this study is the appearances of HFOs, especially FRs, during the latent period in extrahippocampal areas. Although former animal studies have described the presence of FRs during epileptogenesis in the intrahippocampal and parahippocampal areas,^{5,6,14} no studies have examined the extrahippocampal areas during epileptogenesis. However, FRs have been reported in extrahippocampal areas of patients with chronic epilepsy. For instance, Jirsch et al and Urrestarazu et al studied 10 patients with focal seizure and showed the occurrence of HFOs in neocortical epileptogenic regions, as well as in amygdala, MC, and PC FRs.^{17,18} Rektor et al reported thalamic FRs in patients with epilepsy (n = 4) during deep brain stimulation.¹⁹ The appearance of FRs in extrahippocampal areas during the latent period in animals that developed epilepsy could be interpreted to mean that widespread pathological networks contribute to the development of epileptogenesis. The mechanism of occurrence of HFOs in these nonlesioned areas is not clear. It might be a result of the death of a subset of interneurons in distant brain areas due to the generalized seizures of status epilepticus, synaptic reorganization due to propagation of epileptiform activity from the site of injection, or purely functional changes. Further experiments are required to answer these questions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

REFERENCES

1. Jiruska P, Alvarado-Rojas C, Schevon CA, et al. Update on the mechanisms and roles of high-frequency oscillations in seizures and epileptic disorders. *Epilepsia*. 2017;58:1330–9. [PubMed: 28681378]
2. Frauscher B, Bartolomei F, Kobayashi K, et al. High-frequency oscillations: the state of clinical research. *Epilepsia*. 2017;58:1316–29. [PubMed: 28666056]
3. Pitkanen A, Loscher W, Vezzani A, et al. Advances in the development of biomarkers for epilepsy. *Lancet Neurol*. 2016;15:843–56. [PubMed: 27302363]
4. Bragin A, Wilson CL, Engel J Jr. Voltage depth profiles of high-frequency oscillations after kainic acid-induced status epilepticus. *Epilepsia*. 2007;48(suppl 5):35–40.
5. Bragin A, Wilson CL, Engel J Jr. Chronic epileptogenesis requires development of a network of pathologically interconnected neuron clusters: a hypothesis. *Epilepsia*. 2000;41(suppl 6): S144–52. [PubMed: 10999536]
6. Bragin A, Wilson CL, Almajano J, et al. High-frequency oscillations after status epilepticus: epileptogenesis and seizure genesis. *Epilepsia*. 2004;45:1017–23. [PubMed: 15329064]
7. Abdullahi AT, Adamu LH. Neuronal network models of epileptogenesis. *Neurosciences (Riyadh)*. 2017;22:85–93. [PubMed: 28416779]
8. Paxinos G, Watson C. The rat brain atlas in stereotaxic coordinates. New York: Academic Press, 1997; p. 143.
9. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods*. 2004;134:9–21. [PubMed: 15102499]
10. Staba RJ, Wilson CL, Bragin A, et al. Quantitative analysis of high-frequency oscillations (80–500 Hz) recorded in human epileptic hippocampus and entorhinal cortex. *J Neurophysiol*. 2002;88:1743–52. [PubMed: 12364503]
11. Navarrete M, Alvarado-Rojas C, Le Van Quyen M, et al. RIP-PLELAB: a comprehensive application for the detection, analysis and classification of high frequency oscillations in electroencephalographic signals. *PLoS One*. 2016;11:e0158276.
12. Bragin A, Azizyan A, Almajano J, et al. Analysis of chronic seizure onsets after intrahippocampal kainic acid injection in freely moving rats. *Epilepsia*. 2005;46:1592–8. [PubMed: 16190929]
13. Bragin A, Engel J Jr, Wilson CL, et al. Electrophysiologic analysis of a chronic seizure model after unilateral hippocampal KA injection. *Epilepsia*. 1999;40:1210–21. [PubMed: 10487183]
14. Bragin A, Wilson CL, Engel J. Spatial stability over time of brain areas generating fast ripples in the epileptic rat. *Epilepsia*. 2003;44:1233–7. [PubMed: 12919396]
15. Jones RT, Barth AM, Ormiston LD, et al. Evolution of temporal and spectral dynamics of pathologic high-frequency oscillations (pHFOs) during epileptogenesis. *Epilepsia*. 2015;56:1879–89. [PubMed: 26514993]
16. Lévesque M, Bortel A, Gotman J, et al. High-frequency (80–500 Hz) oscillations and epileptogenesis in temporal lobe epilepsy. *Neurobiol Dis*. 2011;42:231–41. [PubMed: 21238589]
17. Jirsch JD, Urrestarazu E, LeVan P, et al. High-frequency oscillations during human focal seizures. *Brain*. 2006;129:1593–608. [PubMed: 16632553]
18. Urrestarazu E, Jirsch J, LeVan P, et al. High-frequency intracerebral EEG activity (100–500 Hz) following interictal spikes. *Epilepsia*. 2006;47:1465–76. [PubMed: 16981862]
19. Rektor I, Dolezalova I, Chrastina J, et al. High-frequency oscillations in the human anterior nucleus of the thalamus. *Brain Stimul*. 2016;9:629–31.

FIGURE 1.

Group comparison of total high-frequency oscillation (HFO; ripple + fast ripple) rates between E- and E+ groups across all periods of recordings. The bar plot of total HFO rate with mean and standard deviations in E- (blue) and E+ (yellow) indicates an overall larger HFO rate in the E+ group during the latent period. *Significant differences ($P < .05$) between latent period (weeks 1–5) and baseline in either the E- (blue) or the E+ (yellow) group. +Significant differences ($P < .05$) between the E- and E+ groups in each recording period

FIGURE 2.

High-frequency oscillation (HFO) events at baseline and during the 5-week latent period. A–D, Demonstration of ripples and fast ripples (FRs) recorded by microelectrodes located in (A) left hippocampus (week 2), (B) thalamus (week 2), (C) motor cortex (week 4), and (D) prefrontal cortex (week 4) in the E+ group. Note that these illustrated ripples and FRs are all superimposed on large spikes (~3–10 Hz, upper plots). Data were presented as raw electroencephalographic events (300 milliseconds, upper plots), events after bandpass filter (100–500 Hz, middle plots), and their time-frequency profiles (bottom plots). A similar pattern was observed throughout the latent period in the E+ group. E, F, Demonstration of HFO events in the 8 recorded areas for the E– group and E+ group, with the illustration of ripples (blue) and FRs (red) as the bin-bars. A 10-minute time frame was chosen from the combination of each animal in each group in each week. After status epilepticus, the lesion site (left hippocampus [LHP]) demonstrated continuous ripples events across all 5 weeks in both groups. Meanwhile, there was an obvious increase and spread of ripples and also FRs in the E+ group in most of the remote recording sites during the entire latent period, but not in the E– group. LMC, left motor cortex; LPC, left prefrontal cortex; LTH, left thalamus; RHP, right hippocampus; RMC, right motor cortex; RPC, right prefrontal cortex; RTH, right thalamus