



Are High-Frequency Activities Reliable Biomarkers of the FCDII Lesion?

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Mouse Model of Focal Cortical Dysplasia Type II Generates a Wide Spectrum of High-Frequency Activities

Chvojka J, Prochazkova N, Rehorova M, Kudlacek J, Kylarova S, Kralikova M, Buran P, Weisssova R, Balastik M, Jefferys JGR, Novak O, Jiruska P. *Neurobiol Dis.* 2024;190:106383. Epub 2023 Dec 17. PMID: 38114051. doi: 10.1016/j.nbd.2023.106383

High-frequency oscillations (HFOs) represent an electrographic biomarker of endogenous epileptogenicity and seizure-generating tissue that proved clinically useful in presurgical planning and delineating the resection area. In the neocortex, the clinical observations on HFOs are not sufficiently supported by experimental studies stemming from a lack of realistic neocortical epilepsy models that could provide an explanation of the pathophysiological substrates of neocortical HFOs. In this study, we explored pathological epileptiform network phenomena, particularly HFOs, in a highly realistic murine model of neocortical epilepsy due to focal cortical dysplasia type II (FCDII). FCD was induced in mice by the expression of the human pathogenic mammalian target of rapamycin (mTOR) gene mutation during embryonic stages of brain development. Electrographic recordings from multiple cortical regions in freely moving animals with FCD and epilepsy demonstrated that the FCD lesion generates HFOs from all frequency ranges, ie, gamma, ripples, and fast ripples up to 800 Hz. Gamma ripples were recorded almost exclusively in FCD animals, while fast ripples occurred in controls as well, although at a lower rate. Gamma-ripple activity is particularly valuable for localizing the FCD lesion, surpassing the utility of fast ripples that were also observed in control animals, although at significantly lower rates. Propagating HFOs occurred outside the FCD, and the contralateral cortex also generated HFOs independently of the FCD, pointing to a wider FCD network dysfunction. Optogenetic activation of neurons carrying mTOR mutation and expressing Channelrhodopsin-2 evoked fast ripple oscillations that displayed spectral and morphological profiles analogous to spontaneous oscillations. This study brings experimental evidence that FCDII generates pathological HFOs across all frequency bands and provides information about the spatiotemporal properties of each HFO subtype in FCD. The study shows that mutated neurons represent a functionally interconnected and active component of the FCD network, as they can induce interictal epileptiform phenomena and HFOs.

Commentary

Focal cortical dysplasia (FCD) is a brain disorder characterized by aberrant cortical development and dysplastic neurons. FCD type II (FCDII), one of the several subtypes of FCD mostly caused by somatic mutations in the mammalian target of rapamycin (mTOR) pathway, is often associated with drug-resistant epilepsy and cognitive and behavioral comorbidities.¹ Although several studies have modeled FCDII in rodents, the electrophysiological features accompanying the epileptic phenotype have not been characterized in detail.

Recently, Chvojka et al² described a wide spectrum of high-frequency activities (HFAs) in an experimental mouse model of FCDII. HFAs encompass a wide variety of oscillatory patterns that include oscillations in the gamma-ripple (45–250 Hz) and fast ripple bands (300–800 Hz). Importantly, the authors found heterogeneity in HFA patterns both within and between experimental animals, which recapitulates the wide diversity of electroencephalogram (EEG) patterns seen in patients.

The authors used in-utero electroporation of a mutated mTOR plasmid to generate FCDII in mice, as previously described.³ The authors first showed that the model recapitulated the histological features of FCDII, including altered neuronal migration, dysmorphic neurons, and abnormal axonal connections.³ Additionally, dysmorphic neurons send axonal processes to the contralateral cortex.⁴ This observation emphasizes how the FCDII lesion can impact remote brain circuits. In addition, the authors captured spontaneous seizures, recorded with chronic EEG in multiple brain regions, in 50% of the electroporated mice. These were characterized by runs of low-amplitude spikes that increase in amplitude but decrease in frequency.² Importantly, the majority of seizures had their onset in the FCDII lesion, demonstrating the epileptogenicity of the dysplastic area.

Interictal epileptiform discharges (IEDs) ranging from isolated spikes to bursts of poly spikes and high-frequency oscillations were recorded in all the FCDII mice. However, the



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algorithm used to detect such activity also identified sharp transient spikes, similar to IEDs, in another cohort of mice that were born following electroporation of a control plasmid. The possibility of nonspecific damage due to in-utero electroporation cannot be ignored. Although there was an overlap in the spectral properties of activity between the experimental groups, the spike rate was higher in FCDII mice. In particular, there was a higher occurrence of HFAs in animals with FCDII, especially in the gamma-ripple and fast ripple bands. The results indicate that FCDII lesions are linked to increased HFAs, which might have an important role in the generation and spread of epileptic seizures.

Gamma-ripple oscillations were also recorded in much higher numbers in the FCDII group compared to the control group, where they were nearly absent. Additionally, the gamma ripples showed peaks in both gamma and fast gamma bands, suggesting the presence of 2 distinct subpopulations of events.² These results suggest that gamma ripples could potentially act as a biomarker to identify the location of epileptogenic tissue and offer important insights for presurgical planning in patients with FCD.

Fast ripples were also observed in both FCDII and control groups but at a much lower frequency in the control group. Previous studies have reported HFAs in the 200–600 Hz frequency band in the somatosensory barrel cortex of rodents during vibrissae stimulation or in response to electrical stimulation of the ventrobasal nuclei of the thalamus.⁵ In this study, fast ripples showed an unimodal distribution of frequency features, with no notable distinctions between FCDII lesional and non-lesional regions. However fast ripple events were more common in the FCDII lesional area than in non-lesional areas, indicating that FCDII lesions play a significant role in producing aberrant HFAs. Previous studies using different mouse models of epilepsy have indicated that HFAs such as fast ripples could act as biomarkers of epileptogenicity, indicating areas in the brain that are capable of triggering seizures.⁶ In the clinic, fast ripples have been proven to be reliable biomarkers for detecting the seizure onset zone compared to IEDs and resecting regions with greater numbers of fast ripples is associated with improved postsurgical outcomes.⁷ Studying the spatiotemporal features of HFAs can provide important information about the mechanisms of seizure genesis and help identify possible targets for surgical treatment.⁸ These findings have profound implications for our understanding of epileptogenesis and offer potential avenues for prognostic stratification and therapeutic intervention in FCDII patients.

Analysis of HFAs outside the FCDII lesion showed the existence of both propagating and independent HFAs, indicating that epileptiform activity due to FCDII can spread beyond the lesion.² The percentage of propagating HFAs was lower compared to HFAs generated independently, suggesting that while FCDII lesions contribute to epileptic network activity, other factors might also influence the generation of HFAs outside the lesion. These findings also corroborate another recent study,⁹ where gene therapy with FCDII mice was highly effective in reducing spontaneous seizures and IEDs. In this study

the gene therapy mainly targeted nondysplastic excitatory neurons around and intermingled with dysplastic neurons in the FCDII lesion, implying that nondysplastic neurons contribute to seizure generation. FCDII mice with or without spontaneous seizures were separated into different experimental groups, both showing IEDs. A comprehensive analysis of HFAs in these groups (seizures vs no-seizures animals) could have been potentially interesting in the Chvojka et al² study. Altogether these findings indicate the involvement of widespread network alterations of brain circuits and suggest an important therapeutic target beyond the focal lesion in suppressing epileptic seizures and mitigating their consequences.¹⁰

One of the intriguing parts of the study by Chvojka et al² was the effect of activation of dysplastic neurons carrying the mTOR mutation using optogenetics. This resulted in the generation of spikes accompanied by HFAs, mostly in the fast ripple band. These findings provide evidence that mutation-carrying neurons have a causal role in the occurrence of HFAs linked to FCD. The resemblance of the frequency traits of optogenetically induced HFAs to spontaneously arising HFAs reinforces the involvement of dysplastic neurons in generating epileptiform activity in FCDII. It remains to be determined whether selective inhibition of these dysplastic neurons would decrease HFAs. Such an experiment would provide more direct insight into the role of these neurons in generating pathological epileptic activity in FCDII.

The study by Chvojka et al² opens new pathways to test and evaluate therapeutic intervention in FCDII. The results of the current study can be used to determine which distinct HFA signatures can act as biomarkers for disease monitoring and assessing treatment response. In order to do so, new studies should focus on understanding the relevance of HFA to the pathological phenotype of FCDII including seizures and behavioral comorbidities.

In conclusion, the findings provided in this study shed light on morphological aspects of FCDII lesions, the occurrence of seizures and interictal activity in FCD-related epilepsy, and the properties of HFAs associated with FCDII. These insights improve our understanding of the pathophysiology and epileptogenesis of FCDII, with implications for diagnosing and treating FCD-related epilepsy. Moving forward, more work is needed to understand the underlying processes of epileptogenesis in FCDII, validate these findings in clinical cohorts, and develop biomarkers to test the efficacy of therapeutic treatments in affected individuals.

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Declaration of Conflicting Interests

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