35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Low Frequency-Modulated High Frequency Oscillations in Seizure-Like Events Recorded from in-vivo MeCP2-Deficient Mice

Sinisa Colic*, Min Lang, Robert G. Wither, James H. Eubanks, Zhang Liang, and Berj L. Bardakjian, *Member, IEEE*

***Abstract*—Rett syndrome is a neurodevelopmental condition caused by mutations in the gene encoding methyl CpG-binding protein 2 (MeCP2). Seizures are often associated with Rett syndrome and can be observed in intracranial electroencephalogram (iEEG) recordings. To date most studies have focused on the low frequencies oscillations (LFOs), however recent findings in epilepsy studies link high frequency oscillations (HFOs) with epileptogenesis. In this study, we examine the presence of HFOs in the male and female MeCP2-deficient mouse models of Rett syndrome and their interaction with the LFOs present during seizure-like events (SLEs). Our findings indicate that HFOs (200-600 Hz) are present during the SLEs and in addition, we reveal strong phase-amplitude coupling between LFOs (6-10 Hz) and HFOs (200-600 Hz) during female SLEs in the MeCP2-deficient mouse model.**

I. Introduction

RETT syndrome is an X-linked neurodevelopmental disorder that primarily affects females[1]. Symptoms typically begin to manifest between 6 and 18 months of age, and may progress to include impairment of cognitive and motor abilities, breathing irregularities, gastrointestinal abnormalities, bone density deficits, social withdrawal and intractable seizures, among others[1].

Mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2)[2] have been found to be the leading cause of Rett Syndrome. Mouse models have been generated that lack Mecp2 function and reproduce clinical features of Rett Syndrome[3]. To date there have been limited intracranial electroencephalography (iEEG) studies done on these mice, with their focus being primarily on the low frequency oscillations (LFOs). These studies have revealed the presence of short, spontaneous epileptiform discharges or SLEs that occur as LFOs[4, 5], however, HFOs have not been studied in the MeCP2-deficient mouse model.

In studies on epilepsy, HFOs have been found to occur during seizures[6], and to be indicators of the seizure onset zone[7]. It has also been shown that as antiepileptic medication was reduced, the occurrence of HFOs increased[8]. Hence, further characterization of differences in HFOs and their cross-frequency coupling may be important in determining the mechanisms of seizure genesis.

In this study, we identify the existence of HFOs and examine cross-frequency interactions of HFOs with LFOs using the modulation index (MI) in order to bridge the gap between epilepsy and Rett.

II. Methods

A. Animal Subjects

All experimental procedures were reviewed and approved by the Canadian Council on Animal Care and local Animal Care Committees. Subjects were derived by crossing female $Mecp2^{+/-}$ mice ($Mecp2^{tm1.1Bird}$, Jackson Laboratory, Bar Harbor, ME) with male wild-type mice described previously [9, 10] to generate experimental subjects of both genders. In total there are (n=8) male $MeCP2^{-/y}$, and (n=5) female $Mecp2^{-/+}$ subjects. All subjects were maintained on a pure C57BL/6 background. While none of the mutant animals were immobile or displayed moribund appearances, each displayed a clear hind limb elevation reflex impairment indicating the presence of Rett-like symptoms[9, 10].

B. iEEG Recording Setup

Male $MeCP2^{-/y}$, and female $Mecp2^{-/+}$ mice were implanted with electrode cap assemblies as described previously[11]. MeCP2-deficient mice were anaesthetized under 2-4% isoflurane through inhalation. Polyimide-insulated stainless steel electrodes were implanted in the hippocampus CA1 (bregma, -2.3 mm; lateral, 1.7 mm; depth, 2.0 mm). A reference electrode was implanted in the frontal cortex (bregma, -3.8 mm; lateral 1.8 mm; depth, 1.5 mm). Female mice were implanted after 250 days of age, which corresponds to the time when symptoms begin to manifest. Male mice were implanted between 40 and 60 days of age, shortly after symptom onset. Mice were given at least 7 days for recovery after surgery before iEEG recordings were taken.

Implanted electrodes were connected to two independent

Manuscript sent April 15, 2013.*Asterisk indicates corresponding author*.

*S. Colic is with the Department of Electrical and Computer Engineering, University of Toronto, Toronto, ON Canada M5S-3G4 (e-mail: sinisa.colic@utoronto.ca)

M. Lang is with the Division of Genetics and Development, University of Toronto Epilepsy Research Program, University of Toronto, Toronto, ON, Canada M5S-1A8

R. G. Wither is with the Department of Physiology, University of Toronto, Toronto, ON, Canada M5S-1A8

J. H. Eubanks is with the Division of Genetics and Development, Department of Surgery, and University of Toronto Epilepsy Research Program, University of Toronto, Toronto, ON, Canada M5S-1A8

L. Zhang is with the Division of Fundamental Neurology, Department of Medicine, and University of Toronto Epilepsy Research Program, University of Toronto, Toronto, ON, Canada M5S-1A8

B. L. Bardkajian is with the Department of Electrical and Computer Engineering, and Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada M5S-3G9

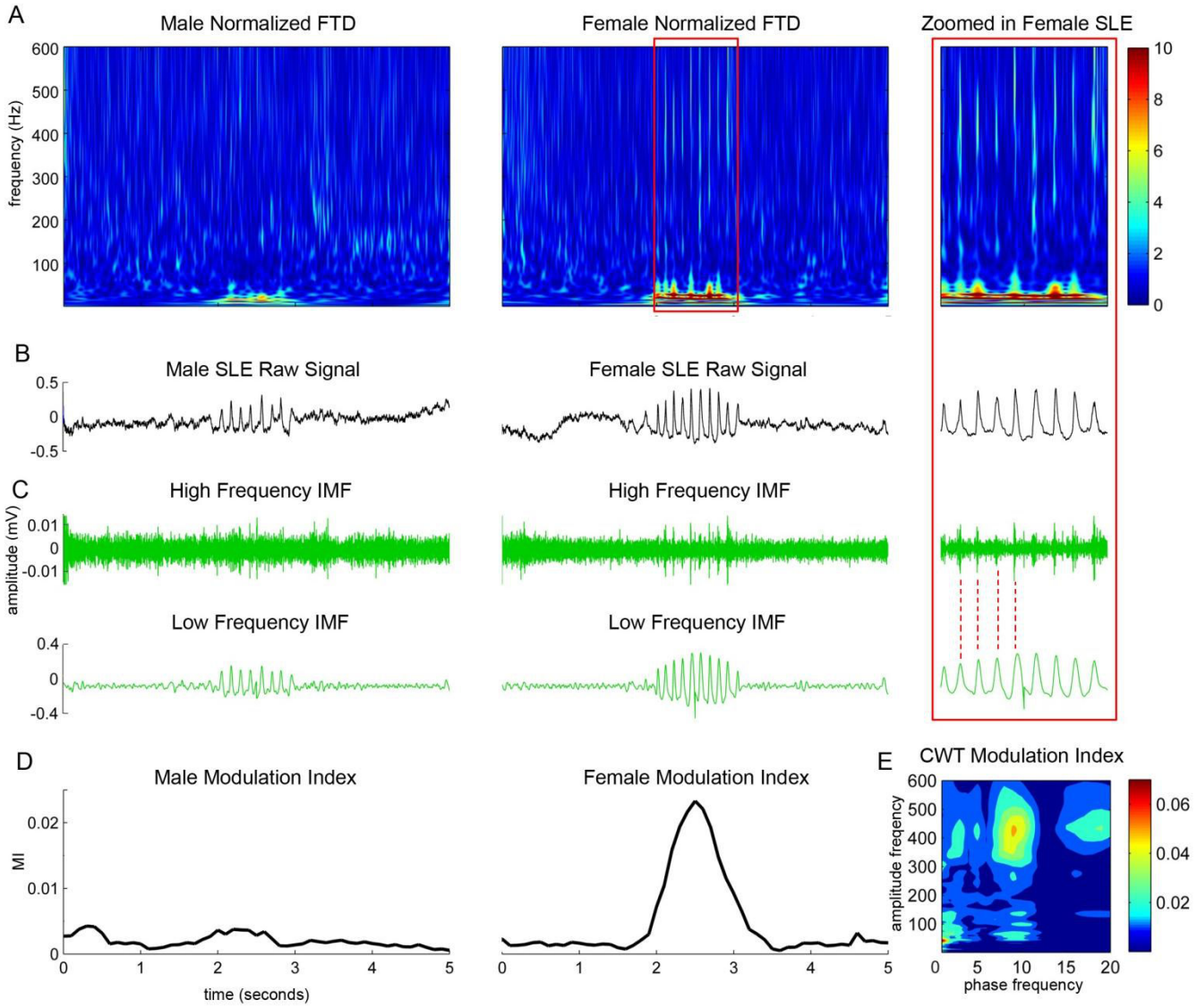

Fig. 1. LFO-HFO modulation in MeCP2-deficient mice A) Normalized frequency time distribution (FTD) shows MeCP2-deficient females have HFO presence in the 200-600 Hz range co-occurring with LFO in the 6-10 Hz range. Red box shows zoomed in portion of a female SLE highlighting the HFOs. B) Raw in-vivo EEG recording for male and female MeCP2-deficient mice with the zoomed in portion to the right. C) The extracted high and low frequency IMFs encompassing the HFO and LFO ranges respectively. The zoomed in portion to the right shows that female HFO bursts are modulated to the peaks of the low frequency. D) LFO-HFO modulation index between the low and high frequency IMFs shows increased modulation in females during an SLE event, and no change for males during the SLE event. E) Modulation Index applied on the zoomed in SLE of the female MeCP2-deficient mouse with signals extracted using continuous wavelet transform (CWT). The result shows LFO-HFO modulation, with LFO phase frequency centered around 7 to 9 Hz and HFO amplitude frequency centered around 350 to 500Hz.

head stages (Model-300, AM Systems, Inc., Carlsberg, WA, USA). The head stages were connected to the electrode caps of the mice using soft wires and female pins. iEEG signals were amplified 1000x, bandpass-filtered (0.01 – 1000 Hz) and digitized (Digidata 1300, Axon Instruments, Weatherford, TX, USA). Data were sampled at 60 kHz and stored using Clampfit 10.2 software (Axon Instruments). Recordings sessions varied from 30 min to 3 h to observe all of the behavior states. Subjects were recorded a minimum of 3 times over a 4-6 week period.

C. iEEG Data Analysis

Data were downsampled to 4k Hz using Matlab 2011a. Large amplitude EMG spikes and 60 Hz power artifacts were removed from the recordings. Segments of large amplitude EMG spikes were removed by using a threshold of 1mV. Two second segments centered on artifact were removed. 60 Hz power and corresponding harmonics were notch filtered using Matlab 2011a's FIR filter with a +/- 0.5 Hz cutoff.

To examine time-frequency characteristics, a continuous wavelet transform (CWT) was applied on 5 seconds of EEG recordings centered on epileptiform discharges to obtain the frequency time distribution (FTD). The wavelet basis function used was Matlab 2011a's cmor6-0.8125 from the Morlet family of functions. The frequencies of interest were 1 to 600 Hz with a 1 Hz step size. To observe simultaneously the high and low frequencies, normalization was applied by dividing the wavelet coefficients along each frequency time-series by the mean of a two second segment prior to an epileptiform discharge.

D. Automated SLE Detections

Automated SLE detection was implemented similarly to what was described previously[4, 12, 13]. The first stage consisted of a 6-10 Hz FIR band pass filter to isolate the frequency range associated with SLEs. The envelope was then obtained by convolving a 200-point aperture with the square of the filtered signal. Using a threshold it was determined which time segments contained SLEs. The threshold was set to five standard deviations from the mean of each recording. Detected SLEs were visually inspected to ensure detected SLEs met conditions of epileptiform discharges outlined previously[4, 5, 14].

E. HFO and LFO Extraction

The HFOs and LFOs were extracted using ensemble empirical-mode decomposition (EEMD)[15]. EEMD unlike conventional filtering does not require band selection a priori, but rather applies an unsupervised decomposition technique to separate a signal into intrinsic mode functions (IMFs). The decomposition is adaptive and dependant on local time characteristics of the data, thus it well suited for nonlinear and nonstationary signals.

In this study, EEMD was employed on non-overlapping time windows of 2.5 seconds, with noise variance of σ^2 = 0.2, and over 100 iterations. This resulted in 13 IMFs, with IMF1 being the highest frequency and IMF13 being the lowest frequency. The HFOs (200 – 600 Hz frequency range) were extracted by the 2nd IMF; meanwhile the LFOs (6 – 10 Hz frequency range) were contained by the 6th and 7th IMFs. Hence, the 6th and 7th IMFs were combined to create the low frequency signal used in the modulation index calculating analysis.

F. Modulation Index

The modulation index (MI) as described previously [16, 17], was used in this study to measure intra-electrode phase-amplitude coupling between the hippocampal LFOs and HFOs. The index was determined as follows: (i) the LFO and HFO bands were extracted from the raw signal using EEMD. The LFO corresponds to the combination of 6th and 7th IMFs and the HFO corresponds to the 2nd IMF; (ii) next, the Hilbert transform was applied to obtain the time series phases of the low frequency signal and time series amplitude envelope of the high frequency signal; (iii) the phases were binned into 20 degree intervals, and the mean of the envelope amplitudes over each phase bin was calculated; (iv) the means corresponding to each bin were normalized by the sum of the means over all bins, transforming the mean amplitude per phase bin into a probability value p_j, where j indicates the phase bin number; (v) an Entropy measure defined by:

$$H = -\sum_{j=1}^{N} p_j \log p_j ,$$

was determined, where N is equal to 18, which corresponds to the number of phase bins; (vi) MI was then obtained by normalizing H:

$$MI = \frac{H_{\max} - H}{H_{\max}},$$

where $H_{\max}$ is the maximum possible entropy value, which for a uniform distribution has a value $H_{\max} = \log N$.

The only difference from the method described above was that we applied a complex continuous wavelet transform (CWT) (Matlab 2011's cmor6-0.8125 basis function) to extract the amplitude and phase signals used to generate figure 1E. Furthermore, in this case it was not necessary to apply Hilbert transform to obtain the phase and amplitude time-series, as the CWT generated complex values for each frequency time-series. The window size was set to one second and was centered on the SLE. The amplitude frequencies of interest were 1 to 600 Hz with a 1 Hz step size. Similarly, the phase frequencies of interest were 1 to 20 Hz with a 1 Hz step size. All other MI results were obtained using EEMD to extract the LFO and HFO signals.

III. Results

Normalized time frequency distribution (FTD) of male and female MeCP2-deficient mice SLEs showed elevated power in the 6-10 Hz (LFO) and 200-600 Hz (HFO) frequency ranges (figure 1A). In addition, the HFO bursts in the females occurred in phase with the LFO spikes as can be seen in the zoomed in portion (figure 1A).

Further analysis using a combination of EEMD to separate LFOs and HFOs (figure 1C), and modulation index to quantify phase-amplitude modulation confirmed coupling of the LFOs and HFOs in female MeCP2-deficient mice during SLEs as seen in figure 1D by the rise in MI value. This trend was seen across all of the female MeCP2-deficient mice.

Complex CWT was applied on a female SLE as a secondary measure of MI (see figure 1E). The results show LFO-HFO modulation, with the strongest coupling occurring between LFO phase frequencies centered between 7 to 9 Hz and HFO amplitude frequencies centered around 350 to 500Hz.

IV. Discussion

In this study we found evidence of HFOs during SLEs in the MeCP2-deficient mouse model of Rett syndrome. This goes in line with recent epilepsy studies which are suggesting HFOs are important markers of seizure genesis and can be used as indicators for seizure onset zones[7].

To better examine HFO interaction in the MeCP2-deficient mouse model, we ran the EEMD to extract HFOs and LFOs, and examined intra-electrode phase-amplitude modulation using the modulation index (MI). The findings revealed phase-amplitude coupling between the LFOs and HFOs during SLEs, with coupling being more pronounced in the female MeCP2-deficient mice than their male counterparts.

Not all SLEs exhibited these modulations, or at the same intensity, and in some SLEs the LFO-HFO modulation was absent. Studies on the human epilepsy model have shown increased HFO presence after medication reduction[8], suggesting that further analysis of HFO interactions may be important in determining the mechanism responsible for seizure generation and termination.

Rett syndrome is a disorder that affects males and females differently[1], and examining the LFO-HFO coupling provides a method to quantify these differences.

Acknowledgment

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and from the Canadian Institutes of Health Research.

References

[1] B. Hagberg, J. Aicardi, K. Dias, O. Ramos, "A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases," Ann. Neurol. Vol. 14, pp. 471-479, 1983.

[2] R.E. Amir, I.B. Van den Veyver, M. Wan, C.Q. Tran, U. Francke, H.Y. Zoghbi, "Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2," Nat. Genet. Vol. 23, (no. 2), pp. 185-188, 1999.

[3] J. Guy, B. Hendrich, M. Holmes, J.E. Martin, A. Bird, "A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome," Nat. Genet. vol. 27, pp. 322-326, 2001.

[4] R.G. Wither, S. Colic, C. Wu, B.L. Bardakjian, L. Zhang, and J.H. Eubanks, "Daily rhythmic behaviors and thermoregulatory patters are disrupted in adult female MeCP2-deficient mice," PLoS ONE, Vol. 7, (no. 4), pp. E35396, 2012.

[5] J.A. D'Cruz, C. Wu, T. Zahid, Y. El-Hayek, L. Zhang, and J.H. Eubanks, "Alterations of cortical and hippocampal EEG activity in MeCP2-deficient mice," Neurobiology of disease, vol. 38, (no. 1), pp. 8-16, Apr 2010.

[6] J.D. Jirsch, E. Urrestarazu, P. LeVan, A. Olivier, F. Dubeau, and J. Gotman, "High-frequency oscillations during human focal seizures," Brain : a journal of neurology, vol. 129, (no. Pt 6), pp. 1593-608, Jun 2006.

[7] J. Jacobs, P. LeVan, R. Chander, J. Hall, F. Dubeau, and J. Gotman, "Interictal high-frequency oscillations (80-500 Hz) are an indicator of seizure onset areas independent of spikes in the human epileptic brain," Epilepsia, vol. 49, (no. 11), pp. 1893-907, Nov 2008.

[8] M. Zijlmans, J. Jacobs, R. Zelmann, F. Dubeau, and J. Gotman, "High-frequency oscillations mirror disease activity in patients with epilepsy," Neurology, vol. 72, (no. 11), pp. 979-986, Mar 2009.

[9] Y. Asaka, D.G.M. Jugloff, L.A. Zhang, J.H. Eubanks, and R.M. Fitzsimonds, "Hippocampal synaptic plasticity is impaired in the Mecp2-null mouse model of Rett syndrome," Neurobiology of disease, vol. 21, (no. 1), pp. 217-227, Jan 2006.

[10] D.G. Jugloff, R. Logan, and J.H. Eubanks, "Breeding and maintenance of an Mecp2-deficient mouse model of Rett syndrome," Journal of neuroscience methods, vol. 154, (no. 1-2), pp. 89-95, Jun 30 2006.

[11] C. Wu, M. Wais, T. Zahid, Q. Wan, and L. Zhang, "An improved screw-free method for electrode implantation and intracranial electroencephalographic recordings in mice," Behavior research methods, vol. 41, (no. 3), pp. 736-41, Aug 2009.

[12] P. Suffczynski, F.H.L. da Silva, J. Parra, D.N. Velis, B.M. Bouwman, C.M. van Rijn, P. van Hese, P. Boon, H. Khosravani, M. Derchansky, P. Carlen, and S. Kalitzin, "Dynamics of epileptic phenomena determined from statistics of ictal transitions," IEEE Transactions on Biomedical Engineering, vol. 53, (no. 3), pp. 524-532, Mar 2006.

[13] S. Colic, R. Wither, J.H. Eubanks, L. Zhang, and B.L. Bardakjian, "EEG analysis for estimation of duration and inter-event intervals of seizure-like events recorded in vivo from mice," Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference, vol. 2011, pp. 2570-3, 2011 2011.

[14] J.-N. Yang, J.-F. Chen, and B.B. Fredholm, "Physiological roles of A(1) and A(2A) adenosine receptors in regulating heart rate, body temperature, and locomotion as revealed using knockout mice and caffeine," American Journal of Physiology-Heart and Circulatory Physiology, vol. 296, (no. 4), pp. H1141-H1149, Apr 2009.

[15] Z. Wu and N.E. Huang, "Ensemble empirical mode decomposition: a noise-assisted data analysis method," Advances in Adaptive Data Analysis, vol. 01, (no. 01), pp. 1-41, 2009.

[16] A.B. Tort, M.A. Kramer, C. Thorn, D.J. Gibson, Y. Kubota, A.M. Graybiel, and N.J. Kopell, "Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task," Proceedings of the National Academy of Sciences of the United States of America, vol. 105, (no. 51), pp. 20517-22, Dec 23 2008.

[17] A.B. Tort, R.W. Komorowski, J.R. Manns, N.J. Kopell, and H. Eichenbaum, "Theta-gamma coupling increases during the learning of item-context associations," Proceedings of the National Academy of Sciences of the United States of America, vol. 106, (no. 49), pp. 20942-7, Dec 8 2009.