Characterization of HFOs in short and long duration discharges recorded from in-vivo Mecp2-Deficient Mice

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

***Abstract*—Mutations in the X-linked gene encoding methyl CpG-binding protein 2 (MeCP2) have been linked to a neurodevelopmental disorder known as Rett syndrome. The disorder is associated with a number of symptoms, of which epileptic seizures are common. In this study we examined the presence of high frequency oscillations (HFOs) and their interactions with low frequency oscillations (LFOs) during epileptiform-like discharges using intracranial electroencephalogram (iEEG) recordings from male and female Mecp2-deficient mice. The study compared differences in mean HFO power levels normalized to baseline along with LFO-HFO modulation observed in short and long duration discharges. Short duration discharges, common to both male and female Mecp2-deficient mice, showed a decrease in mean HFO power levels compared to baseline levels. During the short duration discharges the theta (7-9 Hz) LFOs were found to modulate fast ripple (350-500 Hz) HFOs predominantly in the female Mecp2-deficient mice. Long duration discharges, predominantly observed in male Mecp2-deficient mice, were found to have elevated mean power levels in the ripple (80-200 Hz) and fast ripple (350-500 Hz) frequency ranges when compared to baseline. During the long duration discharges a lower frequency range theta LFO (4-6 Hz) modulated both the ripple (80-200 Hz) and fast ripple (350-500 Hz) HFOs. These findings suggest that the long duration discharges observed in male Mecp2-deficient mice share biomarkers indicative of seizure-like activity.**

I. Introduction

RETT syndrome is a neurodevelopmental disorder linked to mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2)[1]. The clinical manifestation involves impairments of motor and cognitive abilities, breathing irregularities, gastrointestinal abnormalities, social withdrawal and intractable seizures, among others[2]. Rett syndrome is estimated to occur in one out of every 10 000 live female births, whereas with boys it leads to death or an extreme form of mental retardation[3].

Mecp2-deficient male and female mouse models have been generated that recapitulate many of the clinical features associated with Rett syndrome[4]. Due to Mecp2 being an X-linked gene, male Mecp2-deficient mice are more adversely affected than the female Mecp2-deficient mice [4, 5]. Male Mecp2-deficient mice have a shorter lifespan and a greater phenotypic severity score compared to the female Mecp2-deficient mice[6].

Studies employing intracranial electroencephalogram (iEEG) recording devices have shown the presence of spontaneous short (less than 1 sec.) duration epileptiform-like discharges in both male and female Mecp2-deficient mice occurring in the theta frequency band ranging from 6-9 Hz [7, 8]. Long duration discharges (greater than 2 sec.) were commonly seen in the male Mecp2-deficient mice. The majority of iEEG studies on the Mecp2-deficient mice model have focused on low frequency oscillations (LFOs).

High frequency oscillations (HFOs) have only received marginal focus in Rett studies and as a result will be the primary focus in this study. Although HFOs have not been studied extensively on Rett models, they have received a great deal of attention in studies on epilepsy. Previously, it has been shown in clinical trials that HFOs occur during epileptic seizures [9], and are an indicator of the seizure onset zone[10]. Furthermore it has been shown that the level of seizure HFO activity can be affected by changes to antiepileptic medication dosages[11].

In this study we compared differences in mean HFO power along with LFO-HFO modulation index (MI) between short and long duration discharges recorded in male and female Mecp2-deficient mice. Our findings showed presence of increased mean HFO power during long duration discharges, suggesting the long duration discharges are seizure-like in nature.

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 [12, 13] to generate experimental subjects of both genders.

Manuscript sent June 9, 2014.*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

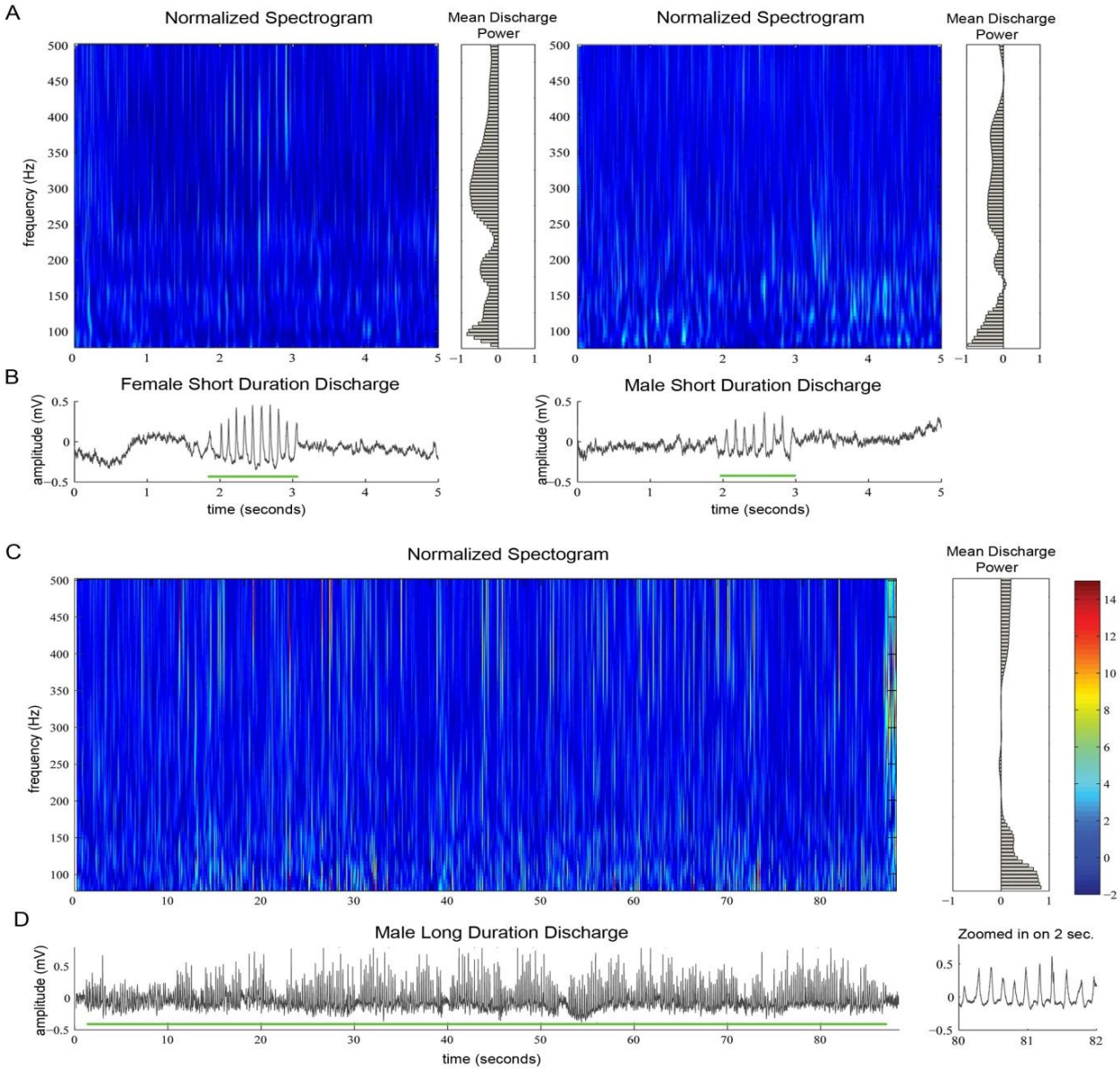

Fig. 1. Comparison of normalized HFO power of short and long discharges in the male and female Mecp2-deficient mouse models of Rett syndrome. A) Normalized spectrogram of the short duration discharges of female (left) and male (right) Mecp2-deficient mice. Shown to the right of each spectrogram is mean HFO power averaged over frequency (80 - 500 Hz) for the duration of the discharges. B) Time-series segments of the short duration discharges of the female (left) and male (right) Mecp2-deficient mice. Green line indicates the duration of the discharge. C) Normalized spectrogram of the male long duration discharge; accompanied by the mean HFO power averaged over frequency for the duration of the discharge. D) Time-series of a long duration discharge observed in a male Mecp2-deficient mice.

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[12, 13].

B. iEEG Recording Setup

Male Mecp2$^{-/y}$, and female Mecp2$^{-/+}$ mice were implanted with electrode cap assemblies as described previously[14]. 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.

C. Normalized Time Frequency Distributions

Data preprocessing was applied as described previously [15]. Time-frequency distributions were examined using the continuous wavelet transform (CWT). The wavelet basis function used was Matlab 2011a's cmor6-0.8125 from the Morlet family of functions. The CWT frequencies of interest ranged from 80 to 500 Hz with a 5 Hz step size. Normalization was achieved using zscore normalization across each wavelet frequency. The zscore mean and standard deviation were determined from a baseline signal approximately two seconds prior to discharge onset.

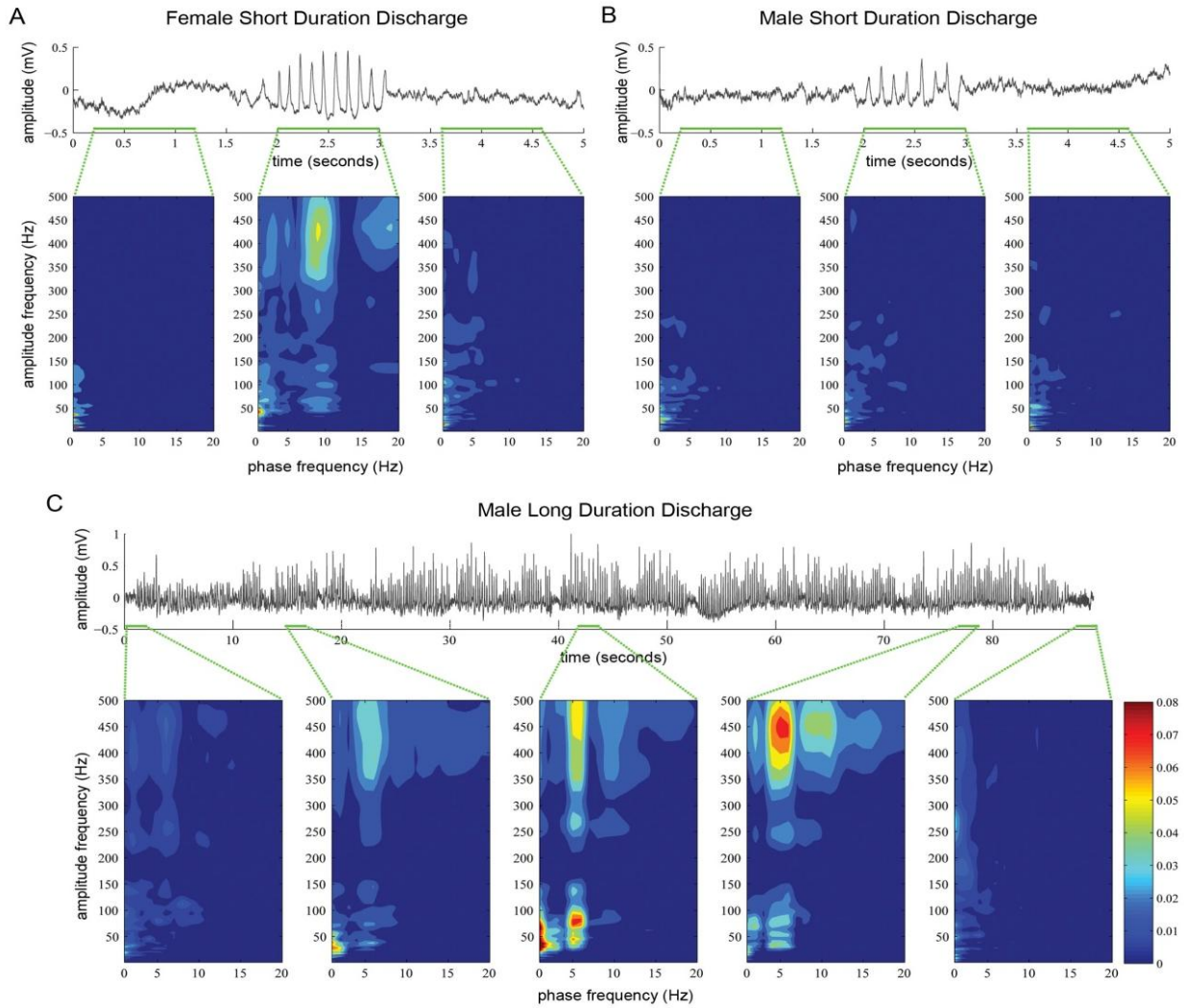

Fig. 2. Comparison of LFO-HFO modulation computed on short and long duration discharges recorded in the male and female mouse models of Rett syndrome. A) Time-series of a short duration discharge observed in a female Mecp2-deficient mouse. The modulation index is shown for three segments: pre, during and post discharge. LFO-HFO modulation is only present during the discharge period. B) Time-series of a typical male short duration discharge with the modulation index computed on segments: pre, during and post discharge shown below. LFO-HFO modulation is absent pre, during and post discharge. C) Time-series of a long duration discharge observed in a male Mecp2-deficient mouse. LFO-HFO modulation was present during the discharge period and absent pre and post discharge.

D. Automated Discharge Detection

Automated discharge detection was implemented similarly to what was described previously[7, 15] with a minor charge to the filtering stage. The first stage (filtering stage) was changed to 4-10 Hz FIR band pass filter to isolate the frequency range associated with the short and long duration discharges observed in the male and female Mecp2-deficient mice. A 200-point aperture was convolved with the square of the filtered signal to obtain the envelope of the filtered signal. Five standard deviations from the mean computed across the entire recording, was used as a threshold for discharge detection. Detected discharges were visually inspected to ensure they met conditions of epileptiform-like discharges outlined previously[7, 8, 16].

E. Modulation Index

The modulation index (MI) was computed using the algorithm proposed by Tort et al. [17, 18], with the filtered phase and amplitude signals obtained from the CWT as described previously[15]. The frequency ranges used for the MI comodulogram plots were 80 to 500 Hz, with 5 Hz step size for the amplitude frequency, and 1 to 20 Hz with 1 Hz step size for the phase frequencies.

III. RESULTS

Comparison of normalized time frequency distribution of short duration discharges of female and male Mecp2-deficient mice shows minor differences in HFO power (figure 1A, B). Both male and female short duration discharges show reduced average power in comparison to the long duration discharges seen in the male Mecp2-deficient mice (figure 1C, D). Specifically, long duration discharges showed an elevation mean power in the ripple (80 - 200 Hz) and fast ripple (350 - 500 Hz) frequency ranges.

Analysis of the modulation index for the short duration discharges of male and female Mecp2-deficient mice showed that males had reduced or no modulation (figure 2B), whereas females had LFO-HFO modulation typically in

the range of 7-9 Hz for the LFO and 350-500 Hz for the HFO (figure 2A). Long duration discharges observed in the male Mecp2-deficient mice showed LFO-HFO modulation with the LFO closer to the delta band, 4 - 6 Hz, modulating the HFO ripple, 80 - 200 Hz and the HFO fast ripple, 350 - 500 Hz frequency ranges (figure 2C).

IV. CONCLUSION

These findings revealed that there exist distinct differences between long duration and short duration discharges. Long duration discharges observed in the male Mecp2-deficient mice show increased mean power in the ripple and fast ripple frequency ranges. In addition, the long duration discharge LFO modulating signal is closer to delta and it modulates both the ripple and fast ripple. Delta modulation of ripple and fast ripple HFOs has previously been documented in seizure state classification [19]. Furthermore increased HFO power in the ripple and fast ripple frequency bands has been identified as a biomarker of epileptic seizures[9, 10]. Hence the presence of elevated power and modulation of HFOs in long duration discharges could be an indication of seizure-like activity in the male Mecp2-deficient mice.

Rett syndrome is a disorder that affects males and females differently[2]. In a recent study it has been shown that treatments applied to male Mecp2-deficient rescue mice has had varied success[6]. Although long duration discharges are more prevalent in male Mecp2-deficient mice, they are not found in all male mice, or in the same concentration.

Future work will examine the prevalence and characteristics of HFOs in long and short duration discharges to assess their viability as biomarkers for treatment outcome.

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

[2] B. Hagberg, J. Aicardi, K. Dias, and 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.

[3] U. Moog, E.E. Smeets, K.E. van Roozendaal, S. Schoenmakers, J. Herbergs, A.M. Schoonbrood-Lenssen, and C.T. Schrander-Stumpel, "Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females," *European Journal of Paediatric Neurology*, vol. 7, (no. 1), pp. 5-12, 2003.

[4] J. Guy, B. Hendrich, M. Holmes, J.E. Martin, and A. Bird, "A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome," *Nature Genetics*, vol. 27, (no. 3), pp. 322-326, Mar 2001.

[5] R.C. Samaco, C.M. McGraw, C.S. Ward, Y. Sun, J.L. Neul, and H.Y. Zoghbi, "Female Mecp2+/− mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies," *Hum Mol Genet*, vol. 22, (no. 1), pp. 96-109, 2013.

[6] M. Lang, R.G. Wither, S. Colic, C. Wu, P.P. Monnier, B.L. Bardakjian, L. Zhang, and J.H. Eubanks, "Rescue of behavioral and EEG deficits in male and female Mecp2-deficient mice by delayed Mecp2 gene reactivation," *Human molecular genetics*, vol. 23, (no. 2), pp. 303-318, 2014.

[7] R.G. Wither, S. Colic, C. Wu, B.L. Bardakjian, L. Zhang, and J.H. Eubanks, "Daily rhythmic behaviors and thermoregulatory patterns are disrupted in adult female MeCP2-deficient mice," *PLoS One*, vol. 7, (no. 4), pp. e35396, 2012.

[8] 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," *Neurobiol Dis*, vol. 38, (no. 1), pp. 8-16, Apr 2010.

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

[10] 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.

[11] 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.

[12] 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," *Neurobiol. Dis.*, vol. 21, (no. 1), pp. 217-227, Jan 2006.

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

[14] 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," *Behav Res Methods*, vol. 41, (no. 3), pp. 736-41, Aug 2009.

[15] S. Colic, M. Lang, R.G. Wither, J.H. Eubanks, Z. Liang, and B.L. Bardakjian, "Low frequency-modulated high frequency oscillations in seizure-like events recorded from in-vivo MeCP2-deficient mice," *Engineering in Medicine and Biology Society (EMBC), 2013 35th Annual International Conference of the IEEE*, pp. 985-988, 2013.

[16] 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.

[17] 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.

[18] 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.

[19] M. Guirgis, Y. Chinvarun, P.L. Carlen, and B.L. Bardakjian, "The role of delta-modulated high frequency oscillations in seizure state classification," *Engineering in Medicine and Biology Society (EMBC), 2013 35th Annual International Conference of the IEEE*, pp. 6595-6598, 2013.