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EEG Analysis for Estimation of Duration and Inter-Event Intervals of Seizure-Like Events Recorded *in vivo* From Mice

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***Abstract*—Rett syndrome is a neurodevelopmental disorder of the brain that affects females more often than males. Its cause is linked to the mutations within the gene encoding methyl CpG-binding protein 2 (MeCP2). Presently, there is little information regarding how the loss of MeCP2 affects brain activity. It has been documented that during awake but immobile state, the MeCP2 deficient mice exhibit spontaneous, rhythmic electroencephalogram (EEG) seizure-like events (SLEs) in the range of 6-9 Hz. In this study, we analyze the cortical EEG activity in female MeCP2-deficient mice over 24 hour recordings. Characterizing the SLE and inter-SLE durations by fitting to a gamma distribution we show similarity to previous *in vivo* epilepsy studies. These results suggest that the SLE and inter-SLE dynamics differ. More precisely, the SLE terminations appear to be a result of time–dependent mechanisms, whereas the inter-SLEs are a result of a random process.**

I. Introduction

RETT syndrome is an X-linked genetic disorder affecting primarily young girls that is caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2) [1]. The clinical syndrome involves impairment of both cognitive and motor abilities, social withdrawal, communication dysfunction, breathing irregularities, and severe intractable seizures [2]. Although MeCP2 is expressed throughout the body, the brain appears to be the organ most affected by the loss of MeCP2 function. The effect on the brain is best seen in electroencephalography (EEG) studies of females with Rett Syndrome revealing the presence of severe intractable seizures. These observations indicate that neural network activity is altered in the Rett syndrome brain. However, the underlying mechanisms responsible for the altered network activity remain to be determined.

Researchers have generated several lines of mutant mice that lack MeCP2, or express a truncated MeCP2 protein, and these mice recapitulate many of the features of clinical Rett syndrome [3][4][5][6]. MeCP2-deficient mouse models serve as instrumental tools for unraveling molecular and cellular events believed to contribute to the deficits seen clinically in females with Rett Syndrome. While in-vitro studies of brain slices found that general properties of MeCP2-deficient neurons were primarily preserved, they also identified an imbalance between excitation and inhibition in different brain regions of MeCP2-deficient mice, and impairments in cortical and hippocampal synaptic plasticity [7][8][9]. To date, however, there have been only limited assessments of neural network activity *in vivo* in mouse models of Rett syndrome. While [5] reported spontaneous bilateral cortical EEG discharges that coincided with myoclonic behavioral convulsions in male Mecp2308/y mice, there have been no studies conducted in female mutants, and no assessments to date of network activity in limbic circuitry.

To begin addressing these issues, we conducted intra-cranial EEG recordings to examine the hippocampal and cortical network activity of female MeCP2-/+ mice [3]. Our results show that spontaneous abnormal rhythmic EEG discharges are observed in somatosensory cortex of MeCP2-deficient mice during the immobile-awake state. Collectively, these results provide the first report of altered EEG activity in male and female MeCP2-deficient mouse models, and illustrate that these mice are prone to spontaneous irregular EEG discharges.

The aim of this study is to further characterize the durations and inter-event intervals of seizure-like events in the *in vivo* mouse model of Rett syndrome and compare them to the characterizations performed on animal *in vivo* and in-vitro models of epilepsy, and human epilepsies. Based on the methods outlined by [10], we applied an automated seizure detection method on 24 hour recordings of six mice, and then we generated histograms of the SLE and inter-SLE durations. The dynamical processes responsible for generating the underlying data were then investigated by fitting a gamma function to the distributions. The gamma distribution was chosen do to its reported goodness of fit to neurological data [11] and prevalence in literature [10].

Characterizing the dynamical properties of the system generating the SLEs in the Rett system will help bridge the commonalities between the SLEs and suggest therapies for treatment of epilepsy found in the Rett syndrome.

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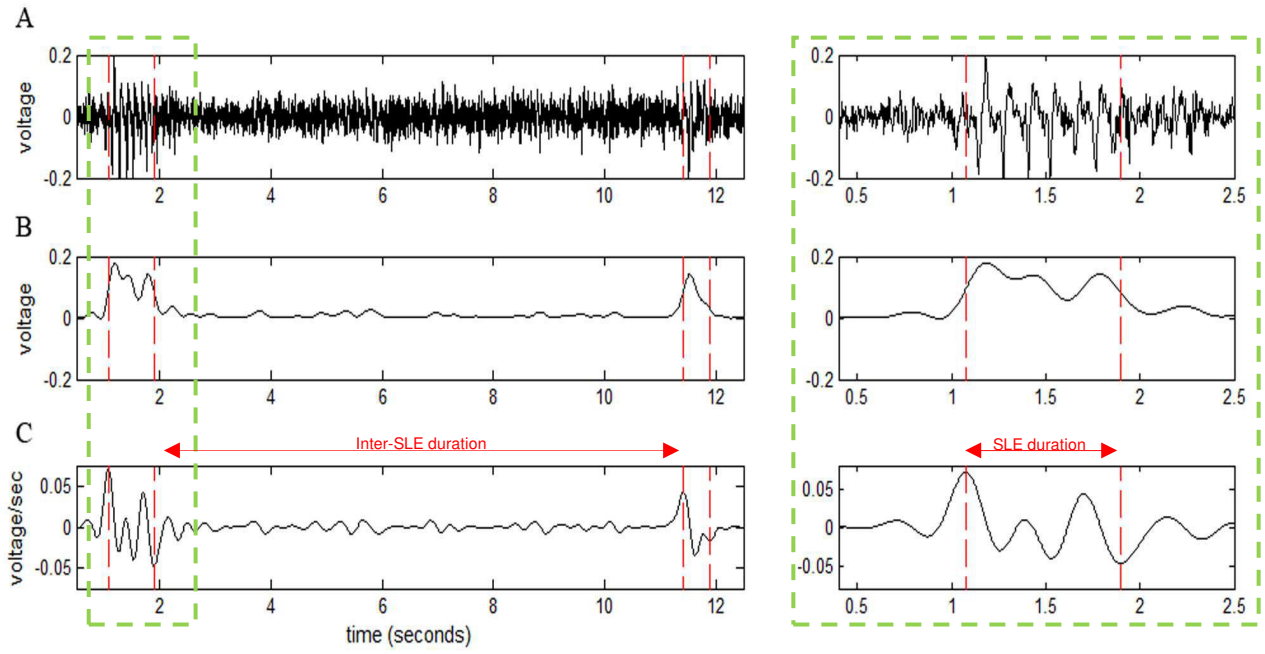

Fig. 1. Automated SLE detection applied on a 12 second EEG segment. a) Raw *in vivo* EEG recording along with the zoomed in portion to the right (green dotted square) focusing on the first SLE. The red dotted line indicates the boundary used to separate between SLE and inter-SLE regions. b) The envelope used to distinguish between SLE and inter-SLE regions. c) The envelope rate of change showing how the inflection points were used to identify the beginning and end of a SLE.

II. Methods

A. Animal Experiments

The experimental animals were derived from six female MeCP2+/- mice [3] (MeCP2tm1.1Bird, Jackson Laboratory, Bar Harbor, ME). Subjects were generated by crossing female MeCP2+/- mice with male wild-type mice as described previously [8][12], and were maintained on a C57BL/6 background. The mutant mice ranged from 65-85 days at age, and 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 [8][12]. All experimental procedures were reviewed in accordance with guidelines established by the Canadian Council on Animal Care, and were approved by local Animal Care Committees before being implemented.

B. EEG Recording Setup

A data acquisition system (Dataquest A.R.T.) with mouse-specific transmitters (TA11ETA-F10, Data Science International, St. Paul, MN, USA) was used for recording electroencephalogram (EEG). Female MeCP2-/+ were operated on for implantation of wireless telemetry probes based on a surgical procedure modified from [13]. The electrode wires were implanted into the somatosensory cortex (bregma -0.6mm, lateral 1.5mm, and depth 1mm) and the contralateral frontal lobe to be used as a reference (bregma +1.2mm, lateral 2.0mm, and depth 0.5mm). Briefly, the animal was anaesthetized with 2% isoflurane and the ventral abdominal wall was opened to place the transmitter (including a battery) in the peritoneal cavity. For EEG, sensing and reference wires connecting the transmitter were orientated rostrally towards the head via a subcutaneous route. EEG recordings were taken over a 24 hour period. The transmission rate of the data was at 250Hz, with digitization rates of 2000Hz. Animals were allowed to recover at least one week from surgery before EEG recordings were taken, and all EEG recordings were obtained while the animals were in their natural behavioral state. The MeCP2-deficient mice displayed the hind-limb clasp impairment when elevated by their tail [3] at the time of implantation, indicating the presence of a Rett-like behavioral impairment at the time of assay. However, none of the MeCP2-deficient mice displayed moribund features such as severely disheveled fur, breath gasping, or prevalent body tremors during the course of the study.

C. Automated SLE Detection

In order to process the 24 hour EEG recordings both efficiently and consistently we developed a straightforward automated SLE detection method. The data was first preprocessed by removing segments indicative of muscle artifacts (characterized by voltages higher than 0.3V), and the 1.25s time period preceding and succeeding the artifacts.

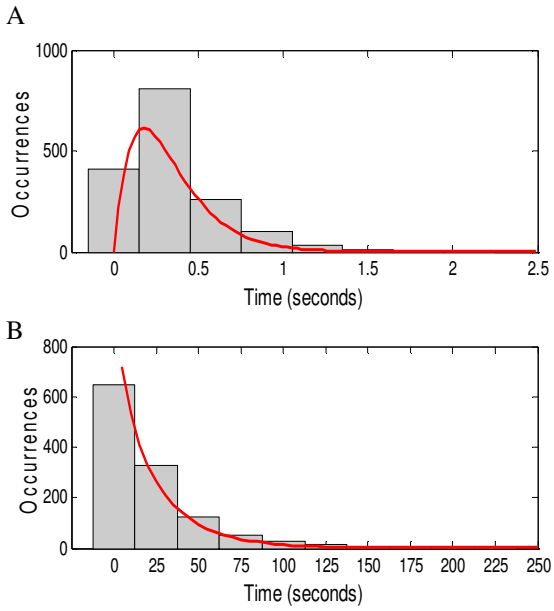

Fig. 2. Gamma distributions (red) and histograms of SLE and inter-SLE durations on 24 hour recording of mouse. a) SLE duration were fit with gamma distribution parameters, α = 2.09 and β = 0.16 . b) Inter-SLE durations were fit with gamma distribution parameters, α = 0.82 and β = 27.88.

TABLE I
SLE AND INTER-SLE DURATIONS ALONG WITH THEIR CORRESPONDING α AND β PARAMETERS TAKEN FROM THE GAMMA FIT WITH 95% CONFIDENCE INTERVAL

SLE			
Animal	α	β	Mean Duration (sec)
1	2.09	0.16	0.34 +/- 0.28
2	2.66	0.09	0.23 +/- 0.22
3	1.78	0.22	0.38 +/- 0.34
4	2.35	0.15	0.34 +/- 0.26
5	1.62	0.21	0.34 +/- 0.37
6	1.64	0.19	0.31 +/- 0.35
Inter-SLE			
Animal	α	β	Mean Duration (sec)
1	0.82	27.88	22.83 +/- 30.23
2	0.71	43.62	31.07 +/- 44.83
3	0.81	71.85	58.33 +/- 71.59
4	0.73	53.2	38.81 +/- 54.60
5	0.69	21.14	14.58 +/- 27.40
6	0.67	29.82	19.97 +/- 31.95

A power spectrum analysis revealed that the SLEs are most prominent in the 6-9Hz band. Therefore we applied a 5-12Hz bandpass filter to isolate the frequency band associated with strong SLEs. As seen in figure 1b, the envelope was produced by convolution of the square of the filtered data with a Gaussian kernel of 200 point aperture peaks when a SLE is present. To further enhance SLE detection, a convolution of the filtered data with the derivative of the Gaussian kernel was applied, yielding the envelope's rate of change.

The seizures were first detected based on their peak value and peak rate of change. A threshold of 0.2 of the peak amplitude was then used in conjunction with inflection points to determine the start and end points of the seizure, and hence the width of SLEs and inter-SLE times. In order to maintain consistency the inter-SLE times were computed only for those segments that lacked artifacts originally. The detection thresholds were maintained the same for all animals. The choice of the peak values and thresholds used for SLE detection was determined in collaboration with physiologists trained to detect seizures.

D. Durations Analysis

The analysis involved fitting the SLE and inter-SLE duration histograms to a gamma distribution,

$$Y = Cx^{\alpha-1}e^{-x/\beta}$$

Where α and β are distribution's shape and scale parameters and C is a normalization constant. The fitting of α parameter was achieved using the gamafit function in the Statistics Toolbox in Matlab 2007b. This function applies a log likelihood approach to find the best parameter fit and gives the maximum likelihood estimation with a 95% confidence interval for α and β parameters. The shape of the Gamma distribution can be separated into three cases. For the case α<1, the distribution has the maximum at the origin and is monotonically decreasing. For α=1, the distribution has an exponential shape. For α>1, the gamma distribution is positively skewed with a zero at the origin and a maximum at some nonzero value.

III. RESULTS

The experimental distributions example from one of the mice (Fig. 2) exposes the difference between SLE and inter-SLE distributions. The SLE distribution is positively skewed, null at the origin and has a maximum at the some non-zero value. By contrast, the inter-SLE distribution with α less than one has a characteristically exponential, monotonically decreasing shape with a longer tail than the SLE distribution and a maximum at 0.

The α parameter of the gamma distributions fitted to the histograms of durations of SLE epochs are given in Table I, second column. It can be noted from the table that the SLE durations have an α that is greater than 1, and range from 1.6 to 2.6, with a confidence of 95%. This is consistent with

findings from other epilepsy studies [10].

The α parameter of the gamma distribution fitted to the histogram of durations of inter-SLE epochs are given in Table I, second column. It can be noted from the table that the SLE durations have an α that is less than 1, and range from 0.67 to 0.82, with a confidence of 95%.

IV. Discussion

SLE epochs have an α parameter greater than one. According to [10], the value of the alpha parameter characteristic to SLE epoch suggests that deterministic time-dependent mechanisms are involved in seizure termination. As well, the probability of terminating a SLE state increases with the amount of time already spent in that state. The fact that the value of alpha for inter-SLE epochs is smaller than one suggests that increasing the time spent in a seizure free state, increases the likelihood of remaining in a seizure free state in the immediate future. This results in clustering of SLE episodes in small regions of time followed by long inter-SLE periods. This seizure clustering is highly pronounced in the Rett syndrome data and has also been documented in other studies [14][15].

Although the SLEs coming from the Rett syndrome and epilepsy are generated by different models, the similarities in the α parameters suggests that the dynamics of the SLE and inter-SLE epochs are analogous with epilepsy; correspondingly the treatments used in treating epilepsy may also be successful in treating Rett syndrome. This preliminary work provides a starting point for future treatments in a system devoid of treatment options.

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