Analyzing Functional Connectivity in the Brain using Cross-Correlation Analysis of Local Field Potentials

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ABSTRACT

Measuring and analyzing local field potential (LFP) signals from basolateral amygdala (BLA), hippocampus (HPC) and medial prefrontal cortex (mPFC) may help understand how they communicate with each other during fear memory formation and extinction. In our research, we have formulated a computationally simple and noise immune instantaneous amplitude cross correlation technique which can deduce lead and lag of LFPs generated in BLA, HPC, and mPFC and the directionality of brain signals exchanged between regions. LFP signals are recorded using depth electrodes in the rat brain and cross correlation analysis is applied to theta wave signals after filtering. We found that rats resilient to traumatic conditions (based on post-stress rapid eye movement sleep (REM)) showed a decrease in LFP signal correlation in REM and non-REM (NREM) sleep cycles between BLA-HPC regions after shock training and one day post shock training compared to vulnerable rats that show stress-induced reductions in REM. It is presumed this difference in neural network behavior may be related to REM sleep differences between resilient and vulnerable rats and may provide clues to help understand how traumatic conditions are processed by the brain.

Keywords: functional connectivity of brain regions; posttraumatic stress disorder (PTSD); medial prefrontal cortex; hippocampus; basolateral amygdala; local field potential; cross correlation of brain signals; multi-site recording; directionality.

1. INTRODUCTION

Understanding functional connectivity in the brain may help detect diseases at an early stage, guide the development of treatments, and also give medical practitioners deeper understanding of brain diseases such as PTSD, epilepsy and schizophrenia [1][2][3]. In this project, we determined electrophysiologically defined sleep and recorded LFPs from the BLA, HPC and mPFC, regions important for fear memory and responding to stress, before and after shock training in a fear conditioning paradigm. We compared signals in vulnerable rats that show stress-induced reductions in REM to those in resilient rats that do not show significant stress-induced reductions in REM. LFP signal data were recorded by taking extreme care to minimize external effects on the signals. These recorded signals were further filtered and processed for finding directionality of signals, cross correlation signals strength and time delay between brain regions. Existing techniques for determining functional connectivity between regions involves computationally complex models like Spike Train analysis using multivariate autoregressive model, Granger Causality and Time Reversal Granger Causality for LFP signals. However, sampling enough spikes from multiple sites is often difficult and recordings can be sensitive to noise [4][5][6]. Some previous studies suggested that LFP signals can be used for estimating the directionality of signals and to find lags in multi-site recordings [4]. Using a cross correlation-based data analysis algorithm helped to derive the analytical lead-lag values and the strength of cross correlation between the signals helped to identify specific differences in resilient and vulnerable rats.

2. METHODOLOGY

2.1 Data Acquisition

The signals were recorded with polyimide insulated wire electrodes attached using a head plug and fine wire cable that was routed through a suspended commutator to allow free movements. Data acquisition was at two times: prior to shock training (baseline) and after shock training. Continuous LFP signals were recorded with sample rate of 2048 samples per second using DataWave™ Technologies software. For sleep state determination, recorded data were hand-scored by a

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trained observer using SleepWave software. Neural activity from BLA, HPC and mPFC was recorded simultaneously to obtain LFPs signals as shown in Figure 1.

Figure 1. Animal recording setup. A schematic of instrumentations for in-vivo neural recording. This includes data an acquisition system, a commutator, and a video recording system.

2.2 Subjects

To perform the experiment, male Wistar rats were obtained from Harlan Laboratories (Frederick, MD) and were kept at Eastern Virginia Medical School. All rats were given a unique identifier that was used to keep track across recording days and conditions including baseline, shock training and NREM and REM sleep. For example, a data file named RAT1BN gives the animal ID: RAT1, experiment day: B-baseline, and sleep state: N-NREM and indicates recordings for RAT1 during baseline and within the NREM sleep stage.

2.3 Surgery

One to two weeks following arrival, the rats were anesthetized with isoflurane (5% induction; 2-3% maintenance) and implanted with skull screw electrodes for recording their EEG and stainless steel wire electrodes were sutured to the dorsal neck musculature for recording their electromyogram (EMG). Bipolar electrodes were implanted into the BLA, HPC and mPFC. Leads from the recording electrodes were routed to a 9-pin miniature plug that mated to one attached to a recording cable. The recording plug was affixed to the skull with dental acrylic and stainless steel anchor screws. Ibuprofen (15 mg/kg) was made available in their water supply 24-48 hours prior to surgery and for a minimum of 72 hours after surgery for relief of post-operative pain. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by Eastern Virginia Medical School's Institutional Animal Care and Use Committee.

2.4 Behavioral Protocol and Animal Testing Setup

After implantation of the electrodes, the animals were permitted to recover for at least two weeks before obtaining a baseline sleep recording. Sleep stages of REM and NREM for animals were monitored in an animal recording set up as shown in Figure 1. For fear conditioning, individual rats were placed in shock chambers (Coulbourn Habitest cages equipped with grid floors (Model E10-18RF) housed in Coulbourn Isolation Cubicles (Model H10-23)) and allowed to freely explore for 5 min. Over the next 20 min, they were presented with 20 footshocks (0.8 mA, 0.5 s duration) at 1.0 min intervals. Shock was produced by Coulbourn Precision Regulated Animal Shockers (Model E13-14) and presented via the grid floor of the shock chamber. Five min after the last shock, the rats were returned to their home cage for sleep recording. The shock chamber was thoroughly cleaned with diluted alcohol (70% EtOH) following each session.

2.5 Independent Component Analysis (ICA)

ICA is a machine learning algorithm that is used to remove unwanted artifacts from signals. After applying ICA, most of the signal artifact gets separated from the mixture of all the signal that has been recorded. ICA for the raw signals was implemented using the MATLAB rica function. Figure 2 shows the raw signals before applying ICA and Figure 3 shows the signals after applying ICA.

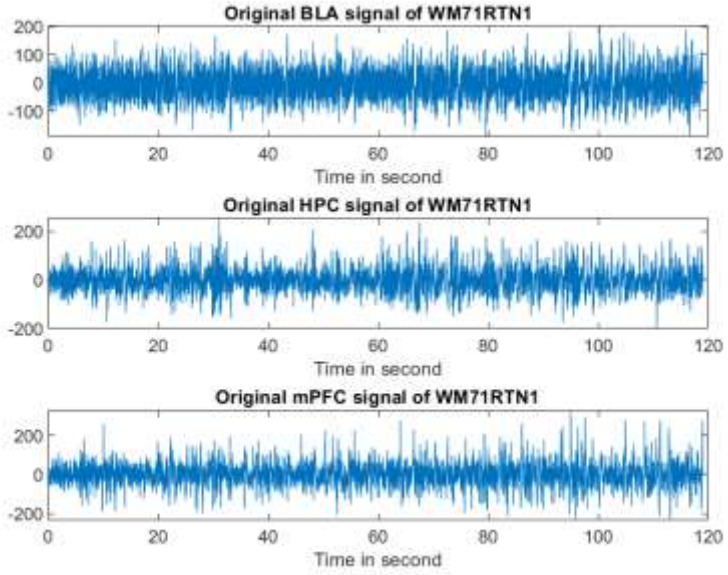

Figure 2. 120 second recordings of raw BLA, HPC and mPFC signals during NREM obtained 7 days post shock training in rat, WM71.

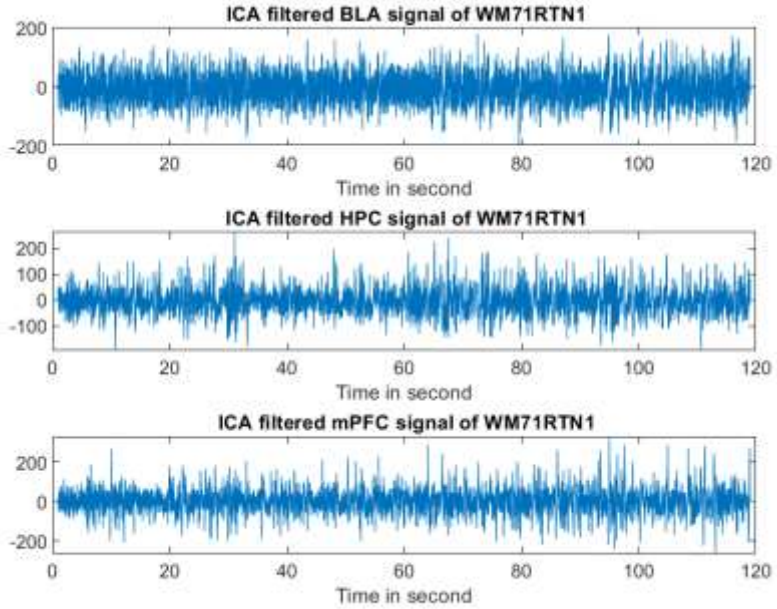

Figure 3. 120 second recordings of ICA filtered BLA, HPC and mPFC signals during NREM obtained 7 days post shock training in rat, WM71.

2.6 Bandpass Filter

After applying ICA, the brain signal independent of nearby brain regions influence was obtained. To obtain theta frequency which ranges from 4.5 Hz to 8 Hz, a band-pass filter was applied. A MATLAB™ bandpass function was used to apply the filter for this range and sample rate of 2048 was provided to function. Theta frequency signals obtained after applying the bandpass filter is been plotted in Figure 4.

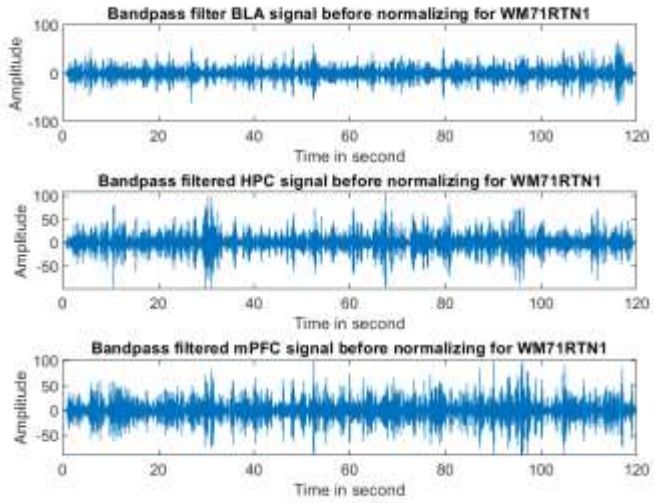

Figure 4. 120 second recordings of bandpass filtered BLA, HPC and mPFC BLA, HPC and mPFC signals during NREM obtained 7 days post shock training in rat, WM71.

2.7 Hilbert Transform

Hilbert transform is used to obtain instantaneous amplitude and phase information of neural signals. After applying the band-pass filter to obtain theta frequency, the instantaneous amplitude was obtained by applying Hilbert transform. The MATLAB™ Hilbert function was used for computing the Hilbert transformation (Figure 5).

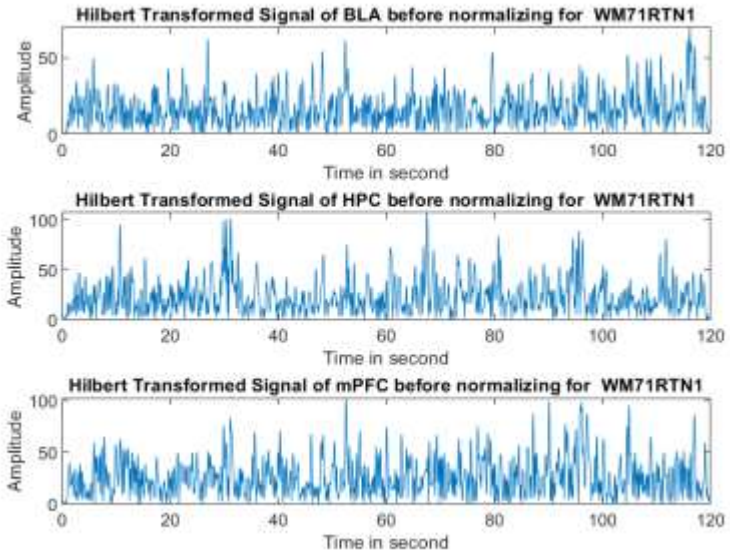

Figure 5. 120 seconds recordings of Hilbert transformed BLA, HPC and mPFC BLA, HPC and mPFC signals during NREM obtained 7 days post shock training in rat, WM71.

2.8 Cross Correlation Analysis

After verifying the algorithm with several data sets, we applied the algorithm for the analysis of neural recordings within sleep using two min time epochs. Each two-min epoch was divided into several time intervals and was analyzed throughout the 120 sec period. This was to find an optimum window where correlation and lead-lag values could be obtained accurately. Using this approach, we found that one sec windows provided the optimum results using the proposed algorithm. Cross-correlation and lead-lag values in the brain regions for before and after shock training were analyzed with the 1 sec time window.

The value of correlation was normalized to ranges between 0 to 1. If the value is 0 or near to zero, it will indicate that the signal was not correlated or loosely correlated. Figure 6 shows examples of highly correlated signals.

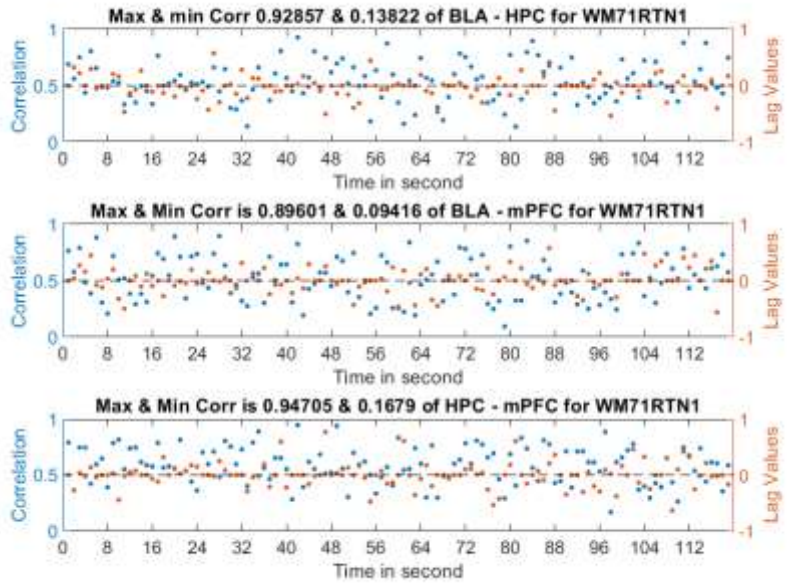

Figure 6. 120 seconds of cross correlation coefficients and time delay values for BLA, HPC and mPFC within NREM at 7 days post shock training in rat, WM71.

3. DISCUSSION AND RESULTS

We choose to study LFP signals over EEG and fMRI signals as they are more immune to volume conduction and noise interference which are major challenges in analyzing functional connectivity of brain regions [7]. A cross-correlation based lead and lag method was found suitable for identifying functional connectivity of brain regions during different sleep stages in rats. As these recordings were recorded at different times, i.e., before and after shock training, it is likely that each recording will have a different noise level in the recorded signal. The cross-correlation based lead and lag method is better at noise handling compared to other existing methods like Granger causality and Partial Directed Coherence (PDC) as these methods are highly sensitive to noise [5]. Existing methods like Granger Causality and PDC are better than the cross-correlation based lead and lag method in calculating directionality, lead and lag time for all frequencies and signals in single analysis at a given time while Cross-correlation based lead and lag method would calculate time delay for pair of signals at a time and would need more steps for calculations. Noise immunity and computation simplicity of cross-correlation based lead and lag method makes it an ideal method for using LFP signals to determine functional connectivity of brain LFP signals within sleep states.

4. CONCLUSION

Implementation of a cross-correlation based data analysis algorithm was found to be effective to assess neural network activities between multiple brain regions. The effects on brain activity due to shock training could be visualized using cross-correlation and lead-lag values. In the future, our aim is to achieve higher accuracy and implement real time data analysis to examine ongoing neural activity within networks.

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REFERENCES

[1] Linda Douw, Marjolein de Groot, Edwin van Dellen, Jan J. Heimans, Hanneke E. Ronner, Cornelis J. Stam, Jaap C. Reijneveld "Functional Connectivity Is a Sensitive Predictor of Epilepsy Diagnosis after the First Seizure" May 26th, 2010, https://doi.org/10.1371/journal.pone.0010839

[2] Zeng LL, Wang H, Hu P, Yang B, Pu W, Shen H, Chen X, Liu Z, Yin H, Tan Q, Wang K, Hu D. "Multi-Site Diagnostic Classification of Schizophrenia Using Discriminant Deep Learning with Functional Connectivity MRI." EBioMedicine. 2018 Apr 30th :74-85. doi: 10.1016/j.ebiom.2018.03.017. Epub 2018 Mar 23. PMID: 29622496; PMCID: PMC5952341.

[3] Zhou, Y., Wang, Z., Qin, L. di, Wan, J. qing, Sun, Y. wen, Su, S. shan, Xu, J. rong. (2012). "Early Altered Resting-State Functional Connectivity Predicts the Severity of Post-Traumatic Stress Disorder Symptoms in Acutely Traumatized Subjects." PLoS ONE, 7(10). https://doi.org/10.1371/journal.pone.0046833Booth, N. and Smith, A. S., [Infrared Detectors], Goodwin House Publishers, New York & Boston, 241-248 (1997).

[4] Adhikari A, Topiwala MA, Joshua AG. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. Neuron 2010;65(2):257–69.

[5] Adhikari, Avishek, et al. "Cross-correlation of instantaneous amplitudes of field potential oscillations: a straightforward method to estimate the directionality and lag between brain areas." Journal of neuroscience methods 191.2 (2010): 191-200.

[6] DeCoteau WE, Thorn C, Gibson DJ, Courtemanche R, Mitra P, Kubota Y, et al. Learning-related coordination of striatal and hippocampal theta rhythms during acquisition of a procedural maze task. Proc Natl Acad Sci USA 2007; 104:5644–9.

[7] B. He, L. Yang, C. Wilke and H. Yuan, "Electrophysiological Imaging of Brain Activity and Connectivity—Challenges and Opportunities," in IEEE Transactions on Biomedical Engineering, vol. 58, no. 7, pp. 1918-1931, July 2011, doi: 10.1109/TBME.2011.2139210.