Nonlinear Analysis of the Hippocampal Subfields of CA1 and the Dentate Gyrus

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Abstract—The paper discusses the use of nonlinear bispectral analysis in examining the hippocampal EEG collected at subfields of CA1 and the dentate gyrus during the vigilance state of REM sleep. The cross-bispectrum and its unique capabilities of detecting and quantifying quadratic nonlinear interactions occurring between these two hippocampal subfields are explained and demonstrated with simulation examples and EEG data. It was found in this study that quadratic nonlinear interactions exist between CA1 and the dentate gyrus in the 6–8 frequency band which dominates the theta (θ) rhythm observed in the hippocampal EEG during REM sleep. As a result, energy components around the frequency band of the second-order harmonics of θ rhythm are not totally spontaneous, but generated largely due to quadratic nonlinear interactions.

I. INTRODUCTION

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VER the years, the EEG has been extensively studied using a great number of signal processing schemes. Most of them are based on the assumption that the EEG is a linear, Gaussian process. However, it is to be noted that, although linear analysis schemes prove to be computationally efficient and useful, they only utilize information retained in the autocorrelation function (i.e., the second-order cumulant). Additional information stored in higher order cumulants (≥3) is therefore ignored in linear analysis of the EEG. For instance, the power spectrum [9] is a very useful tool to determine the energy distribution of a stationary process in the frequency domain, but it cannot distinguish nonlinearly coupled frequencies from spontaneously excited signals with the same resonance condition [13]. The limitation of the power spectrum lies in the fact that it treats a given time series as a stationary Gaussian process consisting of mutually uncorrelated frequency components, and thus fails to notice the difference when frequencies are actually nonlinearly coupled [13].

There is a body of evidence showing that the EEG exhibits non-Gaussian behavior. Elul [7], for example, reported that the EEG of humans involved in the performance of mental arithmetic task exhibits non-Gaussian behavior, while others [1], [17], [18] have reported that the EEG obtained during the vigilance state of slow wave sleep (SWS) has significantly less Gaussian activity than non-SWS EEG, including rapid-eye-movement (REM) sleep. More recently, in our own laboratories, we have found that non-Gaussian activity of the cortical EEG is also vigilance state dependent (SWS > QW > REM sleep), i.e., during SWS, the cortical EEG is associated with more non-Gaussian activity than quiet-waking (QW) and REM sleep [14]. As evidenced from preceding findings, it is important to apply nonlinear signal processing algorithms such as bispectral analysis to address non-Gaussian and nonlinear behavior of the EEG in order to better describe it in the frequency domain.

Studies using bispectral analysis have been conducted by Dumermuth and Barnett [2], [5], [6] in their investigations of the phase interrelationships of the human EEG with subharmonic or harmonic components of alpha (α) rhythm. Ning and Bronzino also used the bispectrum to examine the rat EEG collected during vigilance states of QW, SWS, and REM sleep [14]–[16]. These studies have indicated that the bispectrum is an effective tool to detect and quantify the presence of quadratic phase coupling (QPC), i.e., a phenomenon where two waves interact and generate a third energy component with a frequency equal to the sum of two source frequencies.

In the current study, we use the cross-bispectrum to study quadratic nonlinear interactions occurring between the EEG obtained from two hippocampal subfields CA1 and the dentate gyrus, which have been identified as the primary generators of the θ rhythm observed in the hippocampal EEG during the behavior states of locomotion and REM sleep. Linear coherence analysis [4] of the hippocampal subfields mentioned above have been conducted by many researchers, and results have shown that EEG activities in the frequency band of θ rhythm are strongly correlated between CA1 and the dentate gyrus during REM sleep [3], [8], [10]. Although linear coherence analysis is useful to determine the linear relationship between CA1 and the dentate gyrus, it cannot be used to study nonlinear interactions between these two sites. To our best knowledge, nonlinear analysis of the hippocampal EEG of CA1 and the dentate gyrus has not been performed before. This paper reports our findings derived from the bispectral analysis of the hippocampal EEG.

Section II explains bispectral analysis methods, the bispectrum and cross-bispectrum, and the difference between them. A simulation example is designed to illustrate the unique capabilities of bispectral analysis. Section III discusses data acquisition steps. Results obtained from the study of three animals are summarized and discussed in Section IV.

II. METHODS

The cross-bispectrum has been used in many fields, such as oceanography, geophysics, and passive sonar, to name a few,
to examine the phase relationship between signals collected at different sites [11], [12]. For example, given three zero-mean stationary processes \( \{x_j(n), j = 1, 2, 3\} \), there are two conventional methods, direct and indirect, to compute the cross-bispectrum. Both methods first divide these three processes into \( M \) segments of shorter but equal length. The direct method computes the Fourier transform of each segment for all three processes, and then estimates the cross-bispectrum by taking the average of triple products of Fourier coefficients over \( M \) segments [11], [13], i.e.,

\[
B_{x_1x_2x_3}(\omega_1, \omega_2) = \frac{1}{M} \sum_{m=1}^{M} X_1^m(\omega_1)X_2^m(\omega_2)X_3^m(\omega_1 + \omega_2)
\]

where \( X_j^m(\omega) \) is the Fourier transform of the \( m \)th segment of \( \{x_j(n)\} \) and \(*\) indicates the complex conjugate.

The indirect method computes the third-order cross-cumulant sequence for all segments:

\[
C_{x_1x_2x_3}(k, l) = \sum_{n \in \Gamma} x_1^n(n)x_2^n(n + k)x_3^n(n + l)
\]

(2)

where \( \Gamma \) is the admissible set for argument \( n \). The cross-cumulant sequences of all segments will be averaged to give a resultant estimate:

\[
C_{x_1x_2x_3}(k, l) = \frac{1}{M} \sum_{m=1}^{M} C_{x_1x_2x_3}^m(k, l).
\]

(3)

The cross-bispectrum is then estimated by taking the Fourier transform of the third-order cross-cumulant sequence [11], [13]:

\[
B_{x_1x_2x_3}(\omega_1, \omega_2) = \sum_{k=-\infty}^{\infty} \sum_{l=-\infty}^{\infty} C_{x_1x_2x_3}(k, l) e^{-j(\omega_1 k + \omega_2 l)}.
\]

(4)

While the variance of the estimated cross-bispectrum is inversely proportional to the total number of segments \( M \), the resolution capability is proportional to the length of each segment. Therefore, to compute the cross-bispectrum for processes of finite data length, the number of segments and the length of each segment must be carefully chosen to provide satisfactory results [13].

The cross-bispectrum can be used to examine the cross-QPC occurring between \( \{x_1(n)\} \) and \( \{x_2(n)\} \) and its effects upon \( \{x_3(n)\} \). For example, a peak at \( B_{x_1x_2x_3}(\omega_1, \omega_2) \) suggests that the energy component at frequency \( \omega_1 + \omega_2 \) of \( \{x_3(n)\} \) is generated due to the QPC between frequency \( \omega_1 \) of \( \{x_1(n)\} \) and frequency \( \omega_2 \) of \( \{x_2(n)\} \). In theory, the absence of QPC will generate a flat cross-bispectrum. However, due to finite data length encountered in practice, peaks may appear in the cross-bispectrum at locations where there are no significant cross-QPC. To avoid incorrect interpretation, a cross-bicoherence index can be computed to verify the significance level of cross-QPC. The index is defined below:

\[
bic_{x_1x_2x_3}(\omega_1, \omega_2) = \frac{B_{x_1x_2x_3}(\omega_1, \omega_2)}{\sqrt{P_{x_1}(\omega_1)P_{x_2}(\omega_2)P_{x_3}(\omega_1 + \omega_2)}}
\]

(5)

where \( P_{x_j}(\omega) \) is the power spectrum of process \( \{x_j(n)\} \). The theoretical value of the bicoherence index lies between 0 and 1, ranging from nonsignificant to highly significant.

In situations where the interest is the presence of QPC between two stationary processes, e.g., \( \{x(n)\} \) and \( \{y(n)\} \), and its effects on \( \{x(n)\} \), the cross-bispectrum equations can be modified by replacing \( \{x_1(n)\} \) and \( \{x_2(n)\} \) with \( \{x(n)\} \) and \( \{y(n)\} \), i.e.,

\[
B_{x_1y_2}(\omega_1, \omega_2) = \frac{1}{M} \sum_{m=1}^{M} X^m(\omega_1)Y^m(\omega_2)X^m(\omega_1 + \omega_2).
\]

(6)

To highlight the usefulness of bispectral analysis and the difference between the bispectrum and the cross-bispectrum, the following example was designed to illustrate their unique capabilities:

\[
x_1(n) = e^{j(2\pi f_1(1)n + \phi_1(1))}
\]

\[
x_2(n) = e^{j(2\pi f_2(2)n + \phi_2(2))}
\]

\[
y_1(n) = e^{j(2\pi f_3(1)n + \phi_3(1))}
\]

\[
y_2(n) = e^{j(2\pi f_4(2)n + \phi_4(2))}
\]

\[
x(n) = x_1(n) + x_2(n) + x_1(n)x_2(n)
\]

\[
y(n) = y_1(n) + y_2(n) + y_1(n)y_2(n)
\]

(7)

where \( f_1(1) = f_2(1) = 4 \) Hz, and \( f_2(2) = f_4(2) = 7 \) Hz. To ensure low bias and variance of spectral estimates, 80 segments were generated for both \( \{x(n)\} \) and \( \{y(n)\} \). Each segment was obtained using a sampling frequency of 128 Hz with a duration of 4 s. The initial phases \( \{\phi_1(1), \phi_2(2), \phi_3(1), \phi_4(2)\} \) of each segment were randomly changed (i.e., uniformly distributed between 0 and \( 2\pi \)).

Processes \( \{x(n)\} \) and \( \{y(n)\} \) have three identical dominant frequencies at 4, 7, and 11 Hz (see Fig. 1). However, the 11 Hz of \( \{x(n)\} \) was generated due to the QPC between \( \{x_1(n)\} \) and \( \{x_2(n)\} \) (i.e., 4 Hz and 7 Hz), whereas the 11 Hz of \( \{y(n)\} \) was the result of cross-QPC between \( \{y_1(n)\} \) and \( \{x_2(n)\} \). In other words, the absence of \( \{x_3(n)\} \) would eliminate the 11 Hz frequency of \( \{x(n)\} \) while leaving 11 Hz from \( \{y(n)\} \) as well. These nonlinearities cannot be determined by power spectra because the power spectrum cannot delineate the phase relationship between harmonically related frequencies. This drawback makes the power spectrum inappropriate for nonlinear analysis, but it is not a serious problem for many applications where the energy distribution of the underlying process is the only major concern.

To detect and quantify QPC present within a stationary process \( \{x(n)\} \) or \( \{y(n)\} \), the bispectrum can be used. Fig. 2(a) is the bispectrum of \( \{x(n)\} \). A peak appears at (7 Hz, 4 Hz) and the bicoherence index shows a value of 1, indicating that the 11 Hz frequency is generated due to the QPC between 4 and 7 Hz frequencies of \( \{x(n)\} \). In the bispectrum of \( \{y(n)\} \) (Fig. 2(b)), a smaller peak also appears at the same location, but the bicoherence index shows an insignificant value of 0.04, i.e., the 11 Hz frequency of \( \{y(n)\} \) is not generated by the QPC between 4 and 7 Hz frequencies of \( \{y(n)\} \). However, as described earlier, the 11 Hz of \( \{y(n)\} \) was the result of cross-QPC between \( \{y_1(n)\} \) (4 Hz) and \( \{x_2(n)\} \) (7 Hz). Thus given
two stationary processes, the bispectrum is able to detect and quantify the presence of QPC within each process, but unable to detect the cross-QPC between them.

The cross-bispectrum, on the other hand, can be used to detect the presence of cross-QPC between \( \{x(n)\} \) and \( \{y(n)\} \). As shown in Fig. 3(a), two small peaks appear in \( B_{xyx}(\omega_1, \omega_2) \), with insignificant cross-bicoherence values of 0.04 and 0.14, respectively. The result verifies the generation mechanisms that no frequency component of \( \{x(n)\} \) results from the cross-QPC between \( \{x(n)\} \) and \( \{y(n)\} \). Fig. 3(b) shows the cross-bispectrum \( B_{xyx}(\omega_1, \omega_2) \). The peak at (4 Hz, 7 Hz) has a cross-bicoherence value of 1, meaning that the 11 Hz of \( \{y(n)\} \) is generated due to cross-QPC between the 4 Hz of \( \{y(n)\} \) and the 7 Hz of \( \{x(n)\} \), thus confirming the mechanisms that generate \( \{y(n)\} \).

In this paper, we have employed bispectral analysis to detect and quantify quadratic nonlinearities of the EEG collected from the hippocampal subfields of CA1 and the dentate gyrus from adult rats during the vigilance state of REM sleep.

III. EEG DATA ACQUISITION

Adult Sprague–Dawley rats, i.e., 100–120 days of age, were used in this study. Surgeries were performed under sodium pentobarbital (Nembutal, 55 mg/kg) administered by IP injection. Microelectrodes made from 50 \( \mu \)m nichrome wire or 0.0005 in Teflon-coated stainless steel were stereotaxically implanted in the pyramidal cell layer of the subfield CA1 (AP = −3.8 mm, lat = −2.1 mm, and DV = −2.0 mm below dura) and the granule cell layer of the dorsal blade of the dentate gyrus (AP = −3.8 mm, lat = 2.1 mm, and DV = −2.7 mm below dura). An additional monopolar EEG recording electrode (250 \( \mu \)m, Teflon-insulated, stainless wire)
was positioned in the frontal cortex (AP = 1.0 mm, lat = −0.5 mm, and DV = 0.5 mm) to assist in determining the vigilance state of the animal. Two 1/8 in stainless steel machine screws implanted in the skull served as indifferent and ground electrodes. All electrodes were secured to the skull of the animal with dental cement. Placement of recording electrodes was verified histologically at the conclusion of each experiment.

After at least 48 h of the surgery, animals were placed in an electrically shielded and sound-attenuating recording chamber for a minimum of 1 h acclimation period prior to recording. The hippocampal EEG of the rats was recorded using a Grass model-7 polygraph (bandpass frequency of 0.5–75 Hz). All signals were stored on magnetic tapes using an eight-channel Vetter FM tape recorder (3 dB bandwidth of 0–175 Hz). At the end of acclimation period, EEG activity from the electrodes positioned in region CA1 and the dentate gyrus was continuously recorded for a period of 4 h to allow the animals to cycle through several episodes of various vigilance states of quiet waking, slow wave sleep, and REM sleep. During the recording period, the animal was constantly monitored, and behavioral comments including vigilance state and general activity of the animal were noted on the paper record.

The analog EEG was converted to digital format through an A/D converter at a sampling rate of 128 Hz. Since previous studies had already indicated that correlation between CA1 and the dentate gyrus occurred only during θ activity, we focused our attention on the EEG obtained during REM sleep. Eighty epochs were used to ensure a low variance of bispectral estimates, i.e., approximately 1/80 or 1.25% of its true value [13].

### TABLE I

<table>
<thead>
<tr>
<th>Test</th>
<th>R-1</th>
<th>R-2</th>
<th>R-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subfield</td>
<td>CA1</td>
<td>DG</td>
<td>CA1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DG</td>
</tr>
<tr>
<td>Dominant</td>
<td>7</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>(Hz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>14.5</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>(Hz)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

IV. RESULTS AND DISCUSSION

Power spectra of the hippocampal EEG obtained from CA1 and the dentate gyrus during REM sleep are shown in Fig. 4. Note that there are two dominant frequency bands, i.e., θ (7–7.5 Hz) and its second-order harmonic (14–15 Hz). The dominant frequencies of EEG records collected from three representative animals are summarized in Table I.

Preceding results obtained from power spectra immediately raise two interesting questions regarding the composition of the hippocampal EEG: 1) What is the phase relationship of the θ rhythm between CA1 and the dentate gyrus? and 2) Is the secondary peak caused by nonlinear interactions of θ rhythms?

The linear coherence analysis has been used to describe only the linear relationship between CA1 and the dentate gyrus. As shown in Fig. 5, the magnitude squared coherence (MSC)
TABLE II
COHERENCE FUNCTION OF CA1-DG EEG

<table>
<thead>
<tr>
<th>Test Animal</th>
<th>R-1</th>
<th>R-2</th>
<th>R-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td>7</td>
<td>7.5</td>
<td>7</td>
</tr>
<tr>
<td>MSC</td>
<td>0.79</td>
<td>0.88</td>
<td>0.85</td>
</tr>
<tr>
<td>Phase (deg)</td>
<td>46.5</td>
<td>9.9</td>
<td>37.7</td>
</tr>
</tbody>
</table>

TABLE III
QPC OF THE HIPPOCAMPAL EEG

<table>
<thead>
<tr>
<th>Test Animal</th>
<th>R-1</th>
<th>R-2</th>
<th>R-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>CA1</td>
<td>DG</td>
<td>CA1</td>
</tr>
<tr>
<td>Hip-subfield</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>QPC (Hz, Hz)</td>
<td>7.5, 7.5</td>
<td>7.5, 7.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Bicoherence</td>
<td>0.4</td>
<td>0.66</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Fig. 6. Bispectra of the hippocampal EEG collected at (a) CA1 and (b) the dentate gyrus.

of the hippocampal EEG has a highly significant value in the frequency band of 6–9 Hz. A secondary coherent power also appears in Fig. 5 in the frequency range of 14–16 Hz, with an MSC varying from 0.32 to 0.52 in tested animals. Table II summarizes the MSC and the phase coherence at the dominant frequency of three animals. The largest possible bias and variance in MSC estimate for $N$ epochs data are $1/N$ and $0.296/N$, respectively [4], which are very small for $N = 80$.

High MSC values (0.79–0.88) in the $\theta$ rhythm indicate that $\theta$ is strongly correlated between CA1 and the dentate gyrus. The phase coherence varies in three animals because exact locations of implanted electrodes were different. Although the coherence function can be used to reveal linear relationship, it cannot describe any quadratic phase relationship between $\theta$ rhythms.

Bispectra of the hippocampal EEG are shown in Fig. 6. Quadratic nonlinear interactions between $\theta$ rhythms are obvious with the appearance of sharp peaks in bispectra.

Significance levels of detected QPC are verified using the bicoherence index. Results are summarized in Table III. Significant QPC clearly exists in all three animals, including the EEG collected at the dentate gyrus of animal R-1, although the bicoherence index is 0.4. This observation is consistent with our previous finding that the hippocampal EEG during REM sleep exhibits strong quadratic phase coupling in $\theta$ rhythms [14].

To address the second question raised earlier, cross-bispectra were computed to examine possible cross-QPC between the hippocampal EEG of CA1 and the dentate gyrus. To assure the consistency of results, both direct and indirect methods, described by (1) and (4), were used (Figs. 7 and 8). The appearance of peaks in cross-bispectra suggests that cross-QPC exists between these two hippocampal subfields in the frequency band of $\theta$ rhythms.

In theory, both methods will lead to the same cross-bispectrum when the data length is infinite. However, with
finite data records, direct and indirect methods generally lead to cross-bispectrum estimates with different shapes. Therefore, like power spectrum estimation, users have to choose an appropriate method to extract desired information. Nonetheless, both methods we used here indicate the existence of cross-QPC. Significance levels were estimated using the cross-bicoherence index and are summarized in Table IV.

Based on preceding results obtained from linear and nonlinear analyses, it can be concluded that CA1 and the dentate gyrus are major generators of $\theta$ activities and $\theta$ rhythms are strongly correlated. Moreover, quadratic nonlinear interactions also exist. As observed from bispectra and cross-bispectra, energy components of the hippocampal EEG in the frequency band of the second-order harmonic of $\theta$ (14–15 Hz) are not exclusively spontaneous, but are generated largely due to QPC and cross-QPC of $\theta$ rhythms.

V. CONCLUSION

The bispectrum and the cross-bispectrum were used in this paper to detect and quantify the presence of quadratic nonlinear interactions occurring between the hippocampal EEG collected from CA1 and the dentate gyrus during REM sleep. It was found that the secondary peak energy (14–15 Hz) appearing in the hippocampal EEG of CA1 and the dentate gyrus is generated mainly due to quadratic phase coupling of $\theta$ rhythms (7–7.5 Hz) within and between these two hippocampal subfields. This study demonstrates that the bispectrum and the cross-bispectrum are useful signal processing algorithms in delineating quadratic nonlinearities of bioelectrical events such as the EEG.

REFERENCES

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