



2008 Special Issue

Neuronal population oscillations of rat hippocampus during epileptic seizures

Xiaoli Li^{a,b,*}, John G.R. Jefferys^c, John Fox^c, Xin Yao^b^a Institute of Electrical Engineering, Yanshan University, 066004, China^b Cercia, School of Computer Science, The University of Birmingham, Birmingham B15 2TT, UK^c Department of Neurophysiology, Division of Neuroscience, School of Medicine, The University of Birmingham, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:

Received 20 September 2007

Received in revised form

30 May 2008

Accepted 17 June 2008

Keywords:

Neuronal oscillation

Empirical mode decomposition

Epileptic seizure

Hippocampus

Relaxation oscillator

ABSTRACT

Neuronal population oscillations in the hippocampus have an important effect in the information processing in the brain and the generation of epileptic seizures. In this paper, we investigate the neuronal population oscillations in the hippocampus of epileptic rats *in vivo* using an empirical mode decomposition (EMD) method. A neuronal population oscillation can be decomposed into several relaxation oscillations, which possess a recovery and release phase, with the different frequencies that ranges from 0 to 600 Hz. The natures of relaxation oscillations at the pre-ictal, seizure onset and ictal states are distinctly different. The analysis of relaxation oscillations show that the gamma wave is a lead relaxation oscillation at the pre-ictal stage, then it moves to beta oscillation or theta oscillation while the ictal stage starts; the fast relaxation oscillations are associated with the slow relaxation oscillations in the CA1 or CA3, in particular, the fast relaxation oscillations are associated on the recovery phase of the slow relaxation oscillations during the pre-ictal interval, however move to the release phase of the slow relaxation oscillations during the ictal interval. Comparison of the relaxation oscillations in CA1 and CA3 shows that the neurons in the CA1 are more active during the epileptic seizures than during the pre-ictal stage. These findings demonstrate that this method is very helpful to decompose neuronal population for understanding the underlying mechanism of epileptic seizures.

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Population oscillations are a prominent feature of neural activity of a local neural network. Many evidences supported that through the neuronal population oscillations we may infer the dynamic property of interaction between the cellular and synaptic mechanisms (Buzsaki & Draguhn, 2004). In particular, the synchronization of population oscillations could be considered as a potential mechanism of neural information processing due to it reflects the temporally precise interaction of neural activities (Li, Yao, Jefferys, & Fox, 2007; Wang, 2003; Womelsdorf et al., 2007). An abnormal pattern of synchronization of population oscillation is directly related to the mechanism of neural pathophysiology such as epilepsy and Parkinson disease (Uhlhaas & Singer, 2006). The analysis of neuronal oscillations is very important to tackle the fundamental issues in cognitive processing and neural pathophysiology (Buzsaki & Draguhn, 2004; Engel, Fries, & Singer, 2001; Traub, Jefferys, & Whittington, 1999). However, the neuronal population oscillations are very complicated due to the chemical and electrical interactions (connections) of thousands of neurons *in vivo*, even though a single neuron possesses complex dynamics,

such as resonance and oscillations at multiple frequencies (Llinas, 1988). Briefly, the analysis of the neuronal population oscillations is still a challenging task in the Neuroinformatics.

Wavelet and Fourier transforms become traditional methods to analyze the population oscillations (e.g. Adeli, Zhou, and Dadmehr (2003), Khan and Gotman (2003), Lantz et al. (1999) and Li et al. (2007)). Being derived from the assumption that the population oscillation consists of sinusoidal or wavelet functions (or called basic functions), Fourier transforms can perfectly represent a harmonic oscillation signal, wavelet transforms can present the local information of a non-stationary signal (e.g. Adeli et al. (2003), Khan and Gotman (2003) and Li et al. (2007)). These two transforms depend on the selection of basic functions, and the temporal patterns such as instantaneous amplitude and phase/frequency cannot be accurately estimated (Huang et al., 1998). To overcome the drawback of the Fourier and wavelet methods, an adaptive non-linear decomposition method referred to as Empirical Mode Decomposition (EMD) has been recently proposed (Huang et al., 1998). The idea of this method is based on a simple assumption that any data consists of many simple intrinsic modes of oscillations. Because of the adaptive nature, the EMD method coupling with the Hilbert transform has been shown to better describe temporal patterns in non-stationary non-linear time series in biology (e.g. Balocchi et al. (2004),

* Corresponding author at: Institute of Electrical Engineering, Yanshan University, 066004, China. Tel.: +44 121 414 5142; fax: +44 121 414 2799.

E-mail address: xiaoli.avh@gmail.com (X. Li).

Huang et al. (1998), Li (2006), Liang, Lin, and McCallum (2000) and Wu, Huang, Long, and Peng (2007)). The dynamical mechanism of neuronal population oscillations during epileptic seizure still retains unclear. To obtain the details of neuronal population oscillations is the first step for understanding the mechanisms. The EMD method was used to describe the details of neuronal oscillations in the rat hippocampus during epileptic seizures (Li, 2006). Results demonstrate the advantages of this method, which can break down a complicated signal without a basis function and IMFs embedded in the complicated signal can be described in the time frequency domain. In particular, the amplitude and time of each IMF are not distorted and the IMFs have well behaved Hilbert transforms, the instantaneous frequency and amplitude of each IMF can be accurately calculated. In this study, we will further investigate the details of neuronal population oscillations at the interictal, pre-ictal and ictal states by using EMD, including the description of the neuronal oscillations at the different bands (fast ripple, ripple, gamma and beta waves), and the relations to the generation of epileptic seizures. The description and roles of the neuronal oscillations at the different frequency bands (from pre-ictal to ictal state) will be concerned, such as the lead relaxation oscillation to generate seizures. These detailed analyses of neuronal population oscillation could support us to understand the underlying mechanism of the generation of seizures.

1. Material and methods

1.1. Hippocampal neuronal populations

In this study, the rat tetanus toxin model of focal epilepsy is applied to study the neuronal oscillations in CA1/CA3 of rat hippocampus. Recordings were made during previous studies of this model (Finnerty & Jefferys, 2000, 2002). Methods were described in the previous reports, but briefly, male Sprague-Dawley rats (280–400 g) were anaesthetised with halothane. Bipolar recording electrodes (twisted Teflon-coated stainless steel wire with the tips separated 250–350 μm along the axis of the wires) were placed into CA1 and CA3 of both hippocampi. The location of the electrodes was guided using the evoked response produced by ventral commissural stimulation. One microliter phosphate buffered saline either without (controls) or with 4–5 ng (12 mouse LD_{50}) tetanus toxin (Wellcome Foundation Research Laboratories, Beckenham, Kent, UK) was injected into the right hippocampus (3.5 mm lateral and 2.7 mm posterior to bregma) over 1 min. Then, animals were allowed 24–48 h to recover with free access to food and water.

Recording started before the onset of spontaneous epileptiform discharges. The amplified EEG signal was stored on FM tape (Racal V Store, Racal, Southampton, UK, band-pass DC–3.25 kHz). The data were sampled at 2.5 kHz using Spike2 (CED Ltd, UK) running on a personal computer for further analysis. More detail of the EEG recording can be found in Finnerty and Jefferys (2000, 2002). In this study, the 9 seizures of 6 rats are investigated, namely including 36 recordings (Left CA1/CA3 and right CA1/CA3).

1.2. Empirical mode decomposition

For a given non-stationary signal $s(t)$, the EMD method can decompose the signal as a linear combination of intrinsic mode functions (IMFs), C_n ($n = 1, 2, \dots, N$), N is the number of IMFs. The EMD algorithm (Huang et al., 1998) involves the following steps:

Set $n = 0$ and $s_0(t) = s(t)$. *Step 1:* Define $h_k = s_n(t)$, $k = 0$; *Step 2:* Identification of all the extrema (maxima and minima) of the series $h_k(t)$; *Step 3:* Generation of the upper and lower envelope,

$U(t)$ and $L(t)$, via cubic spline interpolation for all of the maxima and minima, respectively; *Step 4:* Shift. The mean of $U(t)$ and $L(t)$ is denoted by $m_k(t)$ (i.e. $m_k(t) = (U(t) + L(t))/2$), and subtraction of $m_k(t)$ to obtain an IMF candidate $h_{k+1} = h_k - m_k(t)$. The functions $U(t)$ and $L(t)$ should envelop the data between them, i.e. $L(t) \leq h_k(t) \leq U(t)$ for all t . If h_{k+1} is not an Intrinsic mode functions (IMF), then increment k , return to *Step 2* and take $h_{k+1}(t)$ in place of $h_k(t)$; else, define the IMF $C_n(t)$ as $h_{k+1}(t)$ and the residual $s_{n+1}(t) = s_n(t) - C_n(t)$. The IMF should satisfy two conditions: the local maxima of the data are positive, and the local minima are negative and the mean value of the envelopes defined by the local maxima and by the local minima need to be zero for all of the analyzed EEG data. If the convergence criteria are not met, increment n and return to *Step 1*. Typical convergence criteria are to test whether the residual is either smaller than a predetermined value or a monotonic function. At the end of first shift process, we will gain the highest frequency oscillation and the oscillation is extracted from original oscillation. Next shift process will extract the secondary fast oscillation. By repeating the shift process, it will extract the oscillations embedded with different frequencies and a trend data from the original EEG recording.

An example of EMD of EEG recordings is shown in Fig. 1. Using EMD, a set of IMFs, $C_n(t)$ ($n = 1, 2, \dots, N$) can be obtained, as can be seen in Fig. 1B. The EEG data $s(t)$ at the pre-ictal state is decomposed into 9 IMFs (Fig. 1 Ba). These oscillatory modes are almost orthogonal components. The EEG data can be exactly reconstructed by a linear superposition: $s(t) = \sum_{i=1}^N C_i(t) + r_{N+1}(t)$, where $r_{N+1}(t)$ is the residual of the EEG data that presents a trend. The EMD method is a direct extraction of instantaneous amplitude associated with various intrinsic time-frequencies.

1.3. Hilbert transform

The instantaneous phase of an oscillatory time series with narrowband can be obtained by means of the analytic signal concept proposed by Gabor in 1946. Given a time series $x(t)$, the complex process of this time series can be written as $x(t) + ix_H(t) = A(t) \exp(i\varphi(t))$, where the function $x_H(t)$ is the Hilbert transforms of $x(t)$, $x_H(t) = \pi^{-1} P.V. \int_{-\infty}^{\infty} \frac{x(\tau)}{t-\tau} d\tau$, where *P.V.* means that the integral is taken in the sense of the Cauchy principal value. The instantaneous amplitude ($A(t)$) and phase $\varphi(t)$ or frequency ($f(t) = \frac{1}{2\pi} \frac{d\varphi}{dt}$) of each IMF can be gained by above Hilbert transforms. For all of IMFs $C_n(t)$ ($n = 1, 2, \dots, N$) in EEG data, a series of instantaneous amplitudes and frequencies can be obtained, $a_i(t)$ and $f_i(t)$, ($i = 1, \dots, N$). These instantaneous amplitudes and frequencies will be all the details of EEG recordings.

1.4. Detection of relaxation oscillation

After the application of EMD to a neuronal population oscillation, several IMFs can be found in Fig. 1B, and each IMF contains some relaxation oscillations. To analyze properties of these relaxation oscillations, an automated detection method is proposed. Considering the natures of IMF, the procedure of relaxation oscillation detection is the following (seeing Fig. 2): (1) extract the local maxima of a given IMF; (2) interpolate the local maximal points by using a piecewise cubic approximation function of order three, then obtain a upper envelop of the oscillation; (3) detect the local maxima and minima of the upper envelop; (4) detect the relaxation oscillation, which contains two local minimal points of upper envelop and one local maximal point that lies between the two local minimal points. The interval between two minimal points is called the duration of a relaxation oscillation. A relaxation oscillation is composed of two phases: recovery

Fig. 1. (A) The neuronal populations in CA3 at the left hippocampus of epileptic rat from pre-ictal to seizure states (the sampling frequency is 2.5 kHz). The trace contains three typical states: pre-ictal, epileptic seizure onset and ictal. Three short segments from these three different states are indexed as I, II and III for further analysis. (B) The decomposition of three short segments (I, II and III) based on EMD. The empirical mode decomposition of three segments from pre-ictal to ictal states is shown in Ba, Bb and Bc. Several IMFs can be found after EMD operation components. The frequency of these IMFs is from high to low, and these IMFs is composed of relaxation oscillations, the decay of the relaxation oscillation decreases significantly with the increase of the frequency of the relaxation oscillation. By comparison with three decompositions at the different states, a direct finding is that the fast relaxation oscillation are much rich in the ictal state.

and release, the interval between the first minimal point and the maximal point defined as the recovery phase of a relaxation oscillation, the second part of the duration defined as the release phase of a relaxation oscillation. The maximal distance between the upper and lower envelop is defined as the amplitude of a relaxation oscillation. The intervals < 10 ms and > 3 s in duration of a relaxation oscillation are rejected. Several relaxation oscillations from different IMFs appear at the same time, in other words, these relaxation oscillations occur simultaneously. In comparison with the upper envelop of these relaxation oscillations, the first occurrence is called the lead relaxation oscillation.

1.5. The discrimination of relaxation oscillation

Recent findings suggest that the neuronal population oscillation is a mixture of fast oscillations (> 30 Hz) and slow oscillations (< 30 Hz). Very high-frequency oscillations (HFOs) that ranges from 200 to 600 Hz is called fast ripple (Bragin, Engel, Wilson, Fried, & Mathern, 1999), most fast ripples appear with spikes (Engel, Wilson, & Bragin, 2003). The fast oscillation that ranges from 100 to 200 Hz is defined as ripple (e.g. Jirsch et al. (2006) and Worrell et al. (2008)). The wave that ranges from 30 to 100 Hz is called gamma. To calculate the frequency of a relaxation oscillation detected, an adaptive autoregressive (AAR) method (Neumaier & Schneider, 2001) is used in this study. AAR method has an advantage over FFT method in the case of shorter duration EEG data (Guler, Kiyimik, Akin, & Alkan, 2001), which can reduce the spectral losses and give better frequency resolution. The maximal frequency of the relaxation oscillation is referred as the frequency of relaxation oscillation for its classification.

Fig. 2. A typical relaxation oscillation of an IMF within a pre-ictal neuronal population oscillation. The oscillation is composed of two phases (recovery phase and release phase). The duration and amplitude could be used to characterize a relaxation oscillation.

2. Results

2.1. EMD of neuronal populations

In this study, we examine the neuronal populations of rat hippocampal areas CA1 and CA3. The EEG recording (Left CA3) in Fig. 1 describes a typical trace from pre-ictal towards ictal state. To identify the dynamical change of neuronal oscillations at the pre-ictal, seizure onset and ictal state, I-II-III segments are extracted for further analysis. The EMD of three segments are plotted in Fig. 1B. It can be seen that a neuronal population oscillation in the

