ELSEVIER

Journal of Neuroscience Methods 167 (2008) 384–392

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

A semi-automated algorithm for studying neuronal oscillatory patterns: A wavelet-based time frequency and coherence analysis

Rodrigo N. Romcy-Pereira [a,*], Draulio B. de Araujo [b,**], João P. Leite [a], Norberto Garcia-Cairasco [a]

[a] *Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, SP, Brazil*
[b] *Department of Physics and Mathematics, University of São Paulo, Ribeirão Preto, SP, Brazil*

Received 15 May 2007; received in revised form 24 August 2007; accepted 28 August 2007

Abstract

In many experimental designs, animal observation is associated with local field potential (LFP) recordings in order to find correlations between behavior dynamics and neuronal activity. In such cases, relevant behaviors can occur at different times during free-running recordings and should be put together by the time of analysis. Here, we developed a MATLAB semi-automated toolbox to quantitatively analyze the temporal progression of brain oscillatory activity in multiple free-running LFP recordings obtained during spontaneous behaviors. The algorithm works by selecting LFP epochs at user-defined onset times (locked to behavior, drug injection time, etc.), calculates their time–frequency spectra, detects long-lasting oscillatory events and calculates linear coherence between pair of electrodes. As output, it generates several table-like text and tiff image files, besides group descriptive statistics. To test the algorithm, we recorded hippocampus and amygdala LFPs from rats in different behavioral states: awake (AW), sleep (SWS, slow-wave sleep and REMS, rapid-eye movement sleep) and tonic–clonic seizures. The results show that the software reliably detects all oscillatory events present in up to seven user-defined frequency bands including onset/offset time and duration. It also calculates the global spectral composition per epoch from each subject and the linear coherence (with confidence intervals) as a measure of spectral synchronization between brain regions. The output files provide an easy way to do within-subject as well as across-subject analysis. The routines will be freely available for downloading from our website http://www.neuroimago.usp.br/BPT/.

Keywords: Quantitative EEG analysis; Time–frequency analysis; Epilepsy; Sleep; Hippocampus; Amygdala

1. Introduction

Brain oscillatory activity is a property of both local and distributed neural networks found in cortical/sub-cortical circuitries (Cantero and Atienza, 2005). It is commonly generated by the coordinated activity of interconnected excitatory and inhibitory neurons, which produce regular oscillations of field extracellular potentials. Distinct oscillatory patterns are associated to different physiological states (awake, sleep, sedation), perceptual representations (visual, auditory) and cognitive processes (attention, learning and memory) (Cantero and Atienza, 2005; Gross et al., 2001; Steriade, 1997).

The correlation between a neurophysiological measurement and a behavioral response has shown to be a successful and productive approach to investigate the neural networks underlying many behaviors (Jensen et al., 2002; Makeig et al., 2004). However, it is deeply dependent on the availability of objective and quantitative analytical methods to handle and extract the relevant information from the experimental data. It requires automated algorithms to deal with a large amount of recordings that usually contain a high degree of redundancy and eventual artifacts.

The implantation of microelectrodes for local field potential (LFP; or deep EEG) recordings in rodents is a widely used electrophysiological technique to measure the electrical activity state of a brain region and is often used to correlate neural circuitry

Abbreviations: AW, awake; bTFR, binarized time–frequency representation; EEG, electroencephalogram; LFP, local field potential; REMS, rapid-eye movement sleep; STFT, short-time Fourier transform; SWS, slow-wave sleep; TFR, time–frequency representation.

* Corresponding author at: University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP 14049-900, Brazil. Tel.: +55 16 602 2556/4535.
** Corresponding author at: University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP 14049-900, Brazil. Tel.: +55 16 3602 3822/778.
E-mail addresses: rnrpereira@rnp.fmrp.usp.br (R.N. Romcy-Pereira), draulio@biomag.usp.br (D.B. de Araujo).

0165-0270/$ – see front matter
doi:10.1016/j.jneumeth.2007.08.027

activation and behavior. As LFPs and behaviors are intrinsically dynamic variables, they require analytical tools that preserve their temporal information. LFPs are locally recorded and can be partially characterized by their frequency components and associated durations. Similarly, behaviors change with time and therefore, they have duration. However, the animal's phenotypic expression is a qualitative categorical variable.

Different methods can be used to obtain the spectral decomposition of a signal (Drongelen, 2006). In contrast to the traditional Fourier transform, the spectral analysis using the wavelet transform presents several advantages regarding the combined study of the time and frequency domains. Importantly, it presents a better resolution trade-off between the spectral decomposition of LFPs calculated at each time point than the Fourier analog, the Short-Time Fourier Transform (STFT) (Tallon-Baudry et al., 1997). Moreover, the most commonly used quantitative output parameters calculated are the oscillating frequency at a certain time and its spectral power. In many instances when two or more signals are analyzed, the coherence function is calculated to estimate the degree of linear correlation between the signals at specific frequencies (Challis and Kitney, 1991).

Our laboratory has traditionally studied the behavioral expression of epileptic seizures by analyzing the interaction between behavioral components during ictal episodes (Dal-Cól et al., 2006; Garcia-Cairasco et al., 1996). The introduction of the simultaneous recording of LFPs and ictal behaviors through video-EEG recordings prompted us to develop an analytical tool to evaluate the spectral time course of specific behaviors. To put LFPs and behaviors together, we decided to use a time–frequency spectral analysis using the wavelet transform and calculate useful quantitative output parameters such as the sustained frequency (onset, offset and duration) that could be correlated with the annotated behaviors. Some of these parameters had already been used in the literature (Drongelen, 2006). Therefore, we present here an easy to use and freely available algorithm able to extract epochs from multiple full-length files, to help the user to exclude epochs with artifacts and to calculate the time-varying power spectra on a large set of data. The algorithm was tested in well-known datasets whose behaviors were previously determined.

2. Materials and methods

2.1. Algorithm

All routines were written in Matlab 6.5 R13 (Mathworks Inc, MA, USA). The algorithm consists of five steps and routine names are shown in parenthesis.

I Epoch Selection, Extraction and Artifact Rejection (*epoch_select* and *epoch_display*).
II Wavelet Time–Frequency Analysis (*timefreq_sust*).
III Calculation of Sustained Oscillations and Frequency Peaks (*timefreq_sust*).
IV Calculation of Across-Subjects Statistics (*analysis*).
V Calculation of Coherence and Confidence Intervals (*cohereboot*).

2.1.1. Epoch selection, extraction and artifact rejection

The first step of the analysis consists in processing the behavior timetable data. Based on the timetable obtained during the observation of animal's performance, the experimenter will define relevant LFP segments to be analyzed. The routine *epoch_select* reads LFP recordings as text files and generates fixed-length epoch files at user-defined onset times. The routine *epoch_display* displays extracted epochs on the computer screen for visual inspection. By checking all epoch segments on the computer screen, the user can exclude artifact-containing files from the working folder and proceed with the analysis. It is important to stress that epoch extraction is a fully automated task and can be used on multiple files. Artifact rejection has to be done manually.

Input parameters: dataset name, epoch duration, onset times. Output: epoch text files, matlab figure and tiff image with all extracted epochs to be displayed on the computer screen.

2.1.2. Wavelet time–frequency analysis

Time–frequency representation (TFR) of biological signals has important applications in the study of behavioral dynamics. The most intuitive form to simultaneously accomplish time and frequency analysis of a signal is to segment the time series into small fragments (windows) and calculate their spectrum, the so-called Short-Time Fourier Transform (STFT):

$$\mathrm{STFT}(t,\omega)=\int [x(\tau)-W(\tau-t)]\,\mathrm{e}^{-\mathrm{j}\omega\tau}\,\mathrm{d}\tau,$$

where W is the sliding window used to segment the signal.

By computing the STFT of a signal, also known as spectrogram, a power spectrum is obtained for different displacements in time t, segmented by the window W. The spectrogram shows how the energy of the signal is distributed both in time and in frequency and, is typically visualized as an image. In such analysis, the size and type of the chosen window is of fundamental importance. However, as the resolution in time increases (i.e., the length of W decreases), one loses accuracy in the frequency component. Such limitation of the STFT gave rise to the development of alternatives, such as the Wavelet analysis. This method provides a better compromise between time and frequency resolution (Jensen et al., 2002; Tallon-Baudry et al., 1997).

Wavelets are mathematical functions, $\psi(t)$ that satisfy specific requirements. They should (1) decay rapidly as $t\to\pm\infty$, (2) be zero mean, and (3) have a Fourier transform, $\hat{\psi}(\omega)$ that obeys the following condition:

$$\int_0^{+\infty}\frac{|\hat{\psi}(\omega)|^2}{\omega}\,\mathrm{d}\omega=\int_{-\infty}^{0}\frac{|\hat{\psi}(\omega)|^2}{|\omega|}\,\mathrm{d}\omega=C_\psi<+\infty$$

There are many types of wavelets, also known as basis. In this study, we used Morlet wavelet basis for calculating TFRs according to the expression:

$$w(f_0,t)=A_\varphi\cdot \mathrm{e}^{-t^2/2\sigma_t^2}\cdot \mathrm{e}^{\mathrm{i}2\pi f_0 t},$$

where $\sigma_t = 1/2\pi\sigma_f$ is the time of the wavelet and σ_f is its frequency.