Analysis of Rhythmic Patterns Produced by Spinal Neural Networks

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INTRODUCTION

The introduction of the in vitro spinal cord and brain stem spinal cord preparation enabled neuroscientists to extend studies of rhythrogenic networks from the basic organization level to a detailed understanding of the mechanisms involved in pattern generation. Recently the effects of genetic manipulations of known groups of spinal neurons on the function of the locomotor rhythm generator have been assayed by monitoring the motor pattern recorded from spinal neurons and the lumbar ventral roots (Gosgnach et al. 2006; Lanuza et al. 2004; see also reviews by Kiehn 2006; Lev-Tov and O’Donovan 2007). This approach offers great promise for dissecting the internal structure of the generator. However, progress in the field is determined by our ability to reliably detect, analyze, and quantify the output of the pattern-generating circuitry under various experimental conditions. The quantitative analysis of rhythmic patterns produced by the spinal cord is traditionally focused on the frequency and the phase relation between the activities of populations of neurons. The quantitative data are usually extracted from the low-pass filtered or the integrated envelope of rectified rhythmic bursts either manually or semi-manually using a computer-based detection of rhythmic waveforms (see Gosgnach et al. 2006; Lanuza et al. 2004). This type of analysis has several inherent problems: it is inaccurate especially when the signals are attenuated after pharmacological treatments, specific lesions, or genetic manipulations and it does not provide quantitative information about the power of the signal or the precise strength of coupling between populations of neurons. A few studies have attempted to solve these problems by the use of time-series analyses of the rhythmic signals in the time (Kremer and Lev-Tov 1997) and frequency domain (Blivis et al. 2007; Miller and Sigwardt 1998; Straus and Lev-Tov 2003) and thereby estimating automatically the frequency, power, cross-power, phase and mean square coherence of time-series variables. These procedures use uni- and bivariate Fourier analyses which assume stationarity of the rhythmic data. However, most of the rhythmic patterns produced by CPGs exhibit variation with time so that the assumption of stationarity of time-series variables can be applied only to short epochs of data. A possible means by which nonstationary signals can be analyzed is offered in recent years by the short-time Fourier (STFT) and wavelet (WT) transformations. These techniques have been used in a variety of fields including physics (Buresti and Lombardi 1999; van Milligen et al. 1995), geophysics (Grinsted et al. 2004; Maraun and Kurths 2004; Torrence and Compo 1998), biology (reviewed by Lio 2003), and neurobiology (EEG, EMG and single-unit recordings from the CNS) (Kimby et al. 2004; Klein et al. 2006; Lee 2002; Li et al. 2007; Normann et al. 2000; Saab et al. 2005; Wang et al. 2004, 2005; Zhan et al. 2006). In this paper, we describe the use of STFT and WT transformations for automated quantitative analyses of the nonstationary output produced by activation of central pattern generators in the mammalian spinal cord. The cross-Morlet WT followed by coherence analysis was found to be the most useful procedure to determine interrelations between pairs of time series in time and frequency domain. We also describe the use of Monte Carlo simulations against white noise for statistical significance of the cross-wavelet coherence. The use of these procedures to characterize the CPG output in the isolated spinal cord preparations is demonstrated for long duration samples of signals produced by bath application of N-methyl-D-aspartate (NMDA)/S-HT/dopamine (DA) for the dynamic nonlinear pattern produced by the alpha1 adrenoceptor agonist methoxamine and for short-duration nonstationary signal elicited in the spinal cord by electrical stimulation of the brain stem. Our study revealed that the motor rhythms described in the preceding text included hidden components that could be uncovered...
and successfully characterized in the time/frequency domain with the wavelet-based techniques.

METHODS

Stimulation and recordings

In the present study, we used data obtained from wide-band (0.1 Hz to 10 kHz) ventral root recordings in the isolated spinal cord and brain stem spinal cord preparation of the neonatal rat (see Blivis et al. 2007; Gabbay and Lev-Tov 2004; Kremer and Lev-Tov 1997). Data were digitized using an A/D converter and stored on the computer hard disk for subsequent analyses. The data samples are taken from Gabbay and Lev-Tov (2004); and from unpublished results of I. Strauss, D. Blivis, and A. Lev-Tov.

Data conditioning

STFT and WT analyses were performed on the recorded data after high-pass filtering at 40 Hz and rectification. Auto- and cross-spectral analyses were done after low-pass-filtering (5–20 Hz) of the rectified data. All analyses were performed using “Spinalcore,” a Matlab-based statistical software package developed by Mor and Lev-Tov.

Time-series analysis of stationary data

The time-series analysis of stationary data were based on the fast Fourier transform (FFT) (Cooley and Tukey 1965) algorithm in which the signal is decomposed into frequencies and corresponding amplitude coefficients using the equation

$$\text{FT}_x(k) = \sum_{n=1}^{N} x(n)e^{-2\pi i kn/N}$$

Where $N$ is the number of points in the digitized signal $x$, $k = 1, 2, \ldots, N$, is an index for the Fourier transform $\text{FT}_x$, $j = 1, 2, \ldots, N$ is an index for the signal $x$, and $i$ is the basic imaginary unit $\sqrt{-1}$. The power carried by the signal per frequency unit (power spectral density, PSD) was calculated using the Welch’s windows method (Welch, 1967), a window-averaged estimation of the power spectrum

$$P_x(f) = \frac{1}{K \delta} \sum_{i=1}^{K} |\text{FT}_x(i) \cdot \overline{\text{FT}_x^*(i)}|^2$$

Where $f$ is frequency, $T$ is the length of each of the $K$ windows, $k = 1, 2, \ldots, K$, $\text{FT}_x$ is its Fourier transform, and $\overline{\text{FT}_x^*}$ is the complex conjugate. The cross-power spectral density (CPSD) was obtained by replacing the complex conjugate of $\text{FT}_x$ with $\text{FT}_y$

$$P_{xy}(f) = \frac{1}{K \delta} \sum_{i=1}^{K} |\text{FT}_x(i) \cdot \overline{\text{FT}_y^*(i)}|^2$$

This analysis yields a matrix of complex numbers that can be used to extract the power and phase between pairs of time series.

The functional coupling between the two oscillating signals can be evaluated by the magnitude squared coherence estimation (Wang et al. 2004; see also Miller and Sigwardt 1998). Magnitude squared coherence (MSC) function between two jointly stationary stochastic processes $x(t)$ and $y(t)$ can be defined as

$$\gamma_{xy}^2(f) = \frac{|P_{xy}(f)|^2}{P_x(f) P_y(f)}$$

Where $P_x$ and $P_y$ are the PSDs and $P_{xy}$ is the CPSD described in the preceding text.

In the case of nonoverlapping Welch’s windows, a critical level for statistical significance of the MSC can be determined using the independence threshold method (Wang et al. 2004; see also Miller and Sigwardt 1998)

$$E_t = 1 - (1 - \alpha)^{1/4\delta t}$$

Where $K$ is the number of windows and $\alpha$ is the desired level of confidence.

Two more tests for the statistical significance of the coherence were used: the probability of detection and the exact confidence interval (Wang et al. 2004).

The probability of detection is defined as

$$P_a = 1 - P(\gamma \leq E_t|n_s, \gamma_0)$$

Where $P(\gamma_0|n_s, \gamma_0)$ is the cumulative distribution function, $n_s$ is the number of segments, $\gamma_0$ is the coherence described in the preceding text, $\gamma_0^*$ is the estimated coherence (Wang et al. 2004), and $E_t$ is the independence threshold. The exact confidence interval expressed as lower and upper confidence intervals is calculated using an iterative algorithm of the central probability interval of the cumulative distribution function (Wang et al. 2004). As suggested by Wang et al. (2004), the combined use of the three methods described in the preceding text (see Fig. 2) provides a more rigorous approach for the determination of the critical level of MSC.

Time-series analysis of nonstationary data

The algorithm of STFT divides the signal into consecutive overlapping or nonoverlapping windows and repeatedly applies the Fourier transform to each window across the signal

$$\text{STFT}(k,n) = \sum_{i=0}^{L-1} x(i) y^*(i-k) W_{l,n}^{-mi} = \text{STFT}(t,f)|_{t=i \delta t, f = \omega / \delta t}$$

Where $x$ is a digitized signal with a length of $L$, $k = 0, \ldots, L-1, I = 0, \ldots, L - 1$, $y^*(\cdot)$ is a symmetric window [trapezoid (Du and Vuskovic 2004), Hamming, Blackmann], $\delta t$ is the sampling interval, $n$ is the frequency bin, and $W$ is the Fourier kernel.

The cross-STFT analysis of pairs of nonstationary time series was performed by repeatedly applying the CPSD algorithm to each window across the signal (see Fig. 2).

To increase the flexibility of the analysis, we have used WT (see Grinsted et al. 2004; Torrence and Compo 1998; Wang et al. 2005), which decomposes the signal into a set of wavelet coefficients using a 0-mean damped finite function (mother wavelet), localized in time and frequency. The complex Morlet wavelet, used in this study is defined as (Couplaud et al. 1984)

$$\Psi_\eta(\eta) = e^{-\eta^2} e^{i \beta \eta}$$

Where $\eta$ is dimensionless time, $w_0$ is dimensionless frequency, and $i$ is the basic imaginary unit $\sqrt{-1}$. A value of $w_0 = 6$, which has been found to provide the best compromise between the temporal and frequency resolution, was used in our study.

The continuous wavelet transform is the sum over all time of the signal multiplied by scaled, shifted versions of the mother wavelet. This process produces wavelet coefficients that are a function of scale and position

$$W_x^s(s) = \sum_{n=1}^{N} x_n \Psi_\eta \left[ (n' - n) \frac{\delta t}{s} \right]$$

Where $x_n$ is the digitized time series with time steps $\delta t, n = 1, \ldots, N$, $s$ represents scale, and $\Psi$ is the scaled and translated (shifted) mother wavelet. A substantial improvement of computational performance is provided by the convolution theorem explained in Torrence and Compo (1998)

$$W_x^s(s) = \sum_{n=1}^{N} x_n \Psi^s(x_0) e^{i \psi}$$

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RESULTS

We first describe the traditional analysis of rhythmic patterns and then describe the automated analysis that is possible with the new methods that do not require individual bursts to be defined and allow the data to be quantified without arbitrary assumptions. In the remaining part of the paper, we illustrate the power of these methods to identify new features of the locomotor rhythms that cannot be extracted using manual or semi-automated analysis of burst onsets and offset. We do this with reference to three physiological examples. First we show that rhythms induced in sacral motoneurons by a drug cocktail of NMDA/5HT/DA are actually composed of at least two distinct rhythms. A fast rhythm, corresponding to the frequency of bursting, and a previously unreported slow rhythm. Each rhythm responds differently to various experimental interventions.

In another example of the utility of this approach, we examine the sacral rhythms induced by the noradrenergic agonist methoxamine. As with the data shown in Fig. 4, the analysis revealed the presence of two distinct rhythms. The faster rhythm corresponding to the induced bursting, was characterized by a robust left-right alternation and gradually slowed over the course of the recording. A second slower rhythm did not exhibit this slowing and was characterized by lower coherence and variable phase relations.

In the final example, we apply our methods to locomotor-like rhythms induced by a train of stimuli applied to the brain stem. The train of stimuli triggers bursting, but the burst includes evoked responses time-locked to each stimulus. As a result, manual definition of burst onsets and offsets is compromised. Our methods allow the complete dissociation of these responses which occur at distinct frequencies. Furthermore, the analysis reveals that the time-locked evoked events propagate to the sacral cord even though the two ventral funiculi (VF) and one of the ventrolateral funiculi (VLF) are cut, whereas the ipsilateral rhythmic bursts do not.

Traditional analysis of spinal rhythmic patterns

Motor rhythmic patterns can be produced in the in vitro spinal cord preparation of the neonatal rat and mouse by bath application of NMDA/5HT with or without DA. The central pattern generators can also be activated in the absence of drugs by electrical stimulation of sacrocaudal (Lev-Tov et al. 2000; Strauss and Lev-Tov 2003) or lumbar afferents (Marchetti et al. 2001) or by stimulation of the ventromedial medulla (VMM) (Blivis et al. 2007; Zaporozhets et al. 2004). Figure 1 shows rhythmic patterns produced in the isolated spinal cord of the neonatal rat by bath-applied NMDA/5HT/DA and recorded from the left and right ventral roots of the second sacral segment before (control) and after a complete transection of the spinal cord at the lumbo-sacral junction (transected at L6/S1). Analysis of the rhythm is traditionally performed after low-pass filtering or integration of the rectified signals (Fig. 1, rectified LPF 5 Hz). This analysis usually includes measurements of the cycle time (3-1), the left-right phase [(4-1)/(3-1)] and the duty cycle [(2-1)/(3-1)] of the rhythm (Fig. 1, control).

FIG. 1. Population recordings from the left (L) and right (R) ventral roots of the second sacral segment (S2) in the neonatal rat spinal cord before (control) and after transection at the lumbo-sacral junction (transected at L6/S1). Recordings are shown following high-pass filtering at 40 Hz (raw, HPF 40 Hz) and after low-pass filtering of the rectified discharge at 5 Hz (rectified, LPF 5 Hz). The onset and offset of a burst (1, 2), the interval between consecutive bursts (1, 3), and the latency between the onset of LS2 and RS2 bursts (1, 4), are denoted by vertical lines.

\[
\omega_k = \begin{cases} 
2\pi k & : k \leq N/2 \\
N/2i & : k > N/2
\end{cases}
\]

Where \( \xi \) is the Fourier transform of the signal \( x_n \), \( \Psi \) is the Fourier transform of the mother wavelet \( s \), and \( i \) is the basic imaginary unit \( \sqrt{-1} \).

The inter-relation between pairs of time series was studied using cross-WT (XWT) and the WT-coherence (CWT) algorithms. The wavelet cross-spectrum is defined as:

\[
W^{XY} = W^X \Psi^{*} Y W^Y
\]

where \( W^X \) and \( W^Y \) are the auto-wavelets of the two time series and \( * \) denotes complex conjugation. The wavelet coherence between time series was determined using the equation:

\[
R^c(s) = \frac{|S(x^{-1} W^{XY}(s))|^2}{S(x^{-1} W^X(s)) S(x^{-1} W^Y(s))}
\]

Where \( s \) is scale, \( W^{XY}(s), W^X(s), W^Y(s) \) are cross-WT and auto-WT as described in the preceding text and \( S \) is a smoothing operator given in Torrence and Webster (1998).

Statistical significance of the wavelet coherence was tested using the Monte Carlo estimation method based on coherence of 300 pairs of randomly generated white-noise datasets, as described by Grinsted et al. (2004). For convenience, we combined the results of the coherence and cross-spectra to a density plot of the coherent power (see Fig. 3), thereby, omitting noncoherent regions from the cross-wavelet spectrum.

The discontinuity introduced at the end of all data streams by zero padding and a further concomitant power reduction near the edges of the spectra (cone of influence) was calculated and delineated as described in Torrence and Compo (1998). Spectral regions outside the cone are shown in our figures with reduced opacity. For convenience of the readers, the scale units in all the spectrograms shown in the present work have been converted to frequency (Hz) using the conversion formula described in Maraun and Kurths (2004)

\[
1
f = \frac{4 \pi s}{\omega_0 + \sqrt{2 + \omega_0^2}}
\]

Where \( f \) is frequency, \( s \) is scale, and \( \omega_0 \) is dimensionless frequency (\( \omega_0 = 6 \), as explained in the preceding text).
Although such analysis can be readily applied for the control rhythm using either manual or semi-manual computer aided methods, its application to the postlesion data (Fig. 1, transected at L6/S1) is limited due to the difficulty of detecting the exact onset and offset of rhythmic bursts under these conditions. The statistical methods we describe in the following text do not require individual bursts to be defined and therefore allow the data to be quantified without arbitrary assumptions.

We first demonstrate the nonstationarity of long segments of the locomotor-like rhythm using Fourier-based spectral analysis. We then show that STFT can be applied to nonstationary data but that its application with a fixed-sized window involves a compromise between the time and frequency resolution of the data. Finally, we introduce the use of the WT and XWTs, which provide excellent temporal and frequency information, to the analysis of locomotor rhythms under several experimental conditions.

Spectral analysis of rhythmic patterns

Fourier-based spectral analysis of 100-s data sample of the rhythmic pattern produced by bath application of NMDA/SHT/DA is demonstrated in Fig. 2. The signals (Fig. 2A) were rectified (not shown), and the analyses were performed after low-pass filtering of the rectified signals at 5 Hz. All analyses were performed using the mean Welch’s windows technique for uni- and bivariate spectral analysis (METHODS). The results shown in Fig. 2B include the power spectral density (PSD) of each time series and cross-PSD of the two series (CPSD) from which the frequency and mean vector (power and phase) of the complex numbers were extracted for the peak power (mean vector). At the next step, the coherence spectrum of the data were calculated (coherence). The coherence spectrum is shown superimposed with statistical tests for the significance of its peaks (Wang et al. 2004) (see METHODS). Altogether, these analyses show a significant coherence of the ∼0.3-Hz peak and of its second harmonic component.

Analysis of nonstationary rhythmic patterns: windowing

Although the analysis described in the preceding text is straightforward, it has an inherent difficulty; it assumes stationarity of the data, which is generally not true for neural signals produced under different physiological or pathological conditions. The nonstationary nature of the CPG output is evident when longer stretches of data are analyzed (Fig. 2C). In contrast to the 100-s data segment shown in Fig. 2A, a longer (500 s) section of the data exhibits variations in amplitude and frequency that precludes the use of standard Fourier-based analysis. The nonstationarity can be quantified by dividing the data into five consecutive segments and applying Fourier cross-spectral analysis to each of these 100-s segments using the mean Welch’s windows technique with five nonoverlapping windows per segment. The resultant cross-power spectra are shown in a three-dimensional display in Fig. 2D (CPSD). Coherence analysis of these five segments (Fig. 2D, coherence) shows that the main spectral peak (∼0.3 Hz) was highly significant only for the first two 100-s data segments. It was insignificant for the third and fourth segments and was barely significant for the fifth segment. A much slower and smaller peak (∼0.01 Hz) that was evident in the last three data segments did not reach statistical significance in the coherence tests.

STFT approach

A similar windowing strategy is employed by STFT. The STFT algorithm divides the time series into consecutive overlapping or nonoverlapping windows and repeatedly applies the Fourier transform across the signal. The power density (color coded) plots obtained after uni- and bivariate STFT analysis of the same data set are shown in Fig. 2E. However, although the STFT is tailored for analysis of nonstationary signals, it is actually a compromise between the time- and frequency-based views of a signal. A short (10 s) window gives high precision information about the signal in time domain, whereas the information in the frequency domain (especially at its lower range) is limited (Fig. 2E, left). By contrast, a long (400 s) window, provides information about the signal in the frequency domain but no information whatsoever in the time domain (Fig. 2E, right).

The analysis of many neurobiological signals requires high resolution in both the time and frequency domain, which is not possible using STFT with a fixed-sized window in time/frequency domain. For this reason, we chose to apply the continuous WT and thereby construct a higher-resolution time-frequency representation of our signals.

Increasing the flexibility of the analysis in the time/frequency domain: continuous WT

Continuous wavelet analyses of the experiment described in Fig. 2C were performed after high-pass filtering and rectification of the signals. The results of these analyses are shown in Fig. 3A. The continuous WT of the left and right S2 ventral root recording data (WT) provided accurate information about the power (color coded) of the high and low frequencies of the rhythmic signals and on their time localization. The higher-frequency component of the rhythm (∼0.3 Hz) showed gradual increase with time, whereas the slower component was steady at ∼0.01 Hz throughout the sampling time. The XWT shows the interrelation between the two time series in time and frequency domain. Again, two main “shared” frequencies of the time series are clearly evident from the high-power regions (red-brown and yellow-red coding) of the plot. However, although this XWT revealed high-power frequency bands in the time domain, it does not provide information whether the correlation between the two variables at a given frequency and a given time is significant. This information is obtained by using wavelet coherence analysis of the data followed by statistical testing for its significance (see METHODS).

The coherence spectrum of the data is shown in Fig. 3A (wavelet coherence, CWT) after the removal of spectral regions that did not exceed the critical level for statistical significance (calculated using Monte Carlo simulations of 300 series of white noise, see METHODS). This procedure allows extraction of the mean vectors (power and phase) and frequency of specified regions of interest. For convenience, we also combined the results of the coherence and cross-spectra into a density plot of the coherent power (Fig. 3A, coherent power, CXWT, see METHODS).
The phase shifts between the activity of the left and right sides of the cord, calculated from the complex numbers of the matrix within the two regions of interest, are shown superimposed over the coherence spectrum (black arrows), revealing an alternating left-right pattern of both regions. Other quantitative parameters, the frequency and coherence of the time series can be easily extracted from the respective frequency bands of the coherent power and coherence spectra (Fig. 3, B and C). These parameters can be used for further circular and linear statistical inferences.

The rhythm analyzed in Fig. 3 was produced in the sacral segments of the cord by bath applied NMDA/5HT and DA. Such activation requires anatomical continuity between the thoracolumbar and sacrococcygeal segments (Gabbay et al. 2002). We chose to demonstrate the extraction of the quantitative parameters of the rhythm from the wavelet coherence spectra of a study in which we assessed the effects of gradual interruption of the thoracolumbar-sacrococcygeal continuity on the interrelations between the left and right sacral hemicords during NMDA/5HT/DA induced rhythm (Fig. 4). 

Recordings from the left and right S2 ventral roots in the same preparation described in Figs. 2 and 3 are shown before (control), after a bilateral lesion of the ventral funiculus (bilateral VF cut), and after a complete transection of the cord at the
lumbosacral junction (Fig. 4A). The coherence of the ~0.3-Hz frequency band (the faster rhythm, region 1) was not affected significantly by the bilateral VF lesion. However, after the complete transection, the ~0.3-Hz frequency band disappeared, and the spectrum exhibited a number of discrete regions with lower coherence, appearing at different epochs and frequencies (Fig. 4, B and C). The coherence of the slow frequency band (region 2) of the time series declined to ~50% of the control during the first 250 s after the bilateral VF lesion and then (the last 250 s after the VF lesion) did not reach statistical significance (Fig. 4, B and C). Finally, circular statistical analysis of the mean phase revealed a significant alternating left-right pattern of all the regions of interest, before, after the bilateral VF lesion, and after the complete transection (Fig. 4D).

These results reveal and quantify several aspects of the locomotor rhythm under the different conditions. First, they show that the WT and WT coherence analyses clearly detected the dominant alternating left-right ~0.3-Hz rhythm present before and after the bilateral VF cut. In addition, the analysis revealed the presence of a much slower (~0.01 Hz) component of the rhythm that is not readily apparent from visual inspection of the data. Remarkably, this slow component exhibits a similar phase (~180°) to the faster component, thus alternates between the left and right sides of the cord. They also show that transection of the ventral funiculi carrying communicating
axons between the lumbar and sacral cord has little effect on the coherence and phase of the ∼0.3-Hz rhythm but reduces the coherence, alters the phase, and then blocks the slower rhythm. Finally, when the lumbar and sacral cords are separated the sacral rhythm breaks, although brief epochs of alternating activity remain (regions a–d in Fig. 4, B and C). Interestingly, phase analysis of incoherent spectral regions (not shown) located between regions a–d (Fig. 4B, transected at L₆/S₁) also revealed significant alternating directionality (φ L-R = 206 ± 81.2°, n = 115,000) although the r vector was much smaller (rv = 0.36) compared to those of the coherent regions shown in Fig. 4B. This latter finding shows that the phase data by itself cannot be used as a reliable estimator of coupling.

In summary, the slow component of the rhythm induced by NMDA/SHT/DA in the sacral segments originates in the thoracolumbar cord, and it is transmitted caudally by VF axons. The fast rhythm also originates in the thoracolumbar cord, and it drives the sacral networks using descending/propriospinal pathways the axons of which travel caudally through the VLF. The occurrence of epochs with a significant alternating left-right pattern after the complete transection of the cord suggested that the sacral network is rhythmogenic and that the NMDA/SHT/DA cocktail is not its optimal activator.

Wavelet and coherence analysis of rhythmic patterns produced in vitro by bath-applied methoxamine and by stimulation of the VMM

In the next part of the study, we demonstrate the use of WT for the analyses of the nonstationary rhythmic bursting produced in the isolated spinal cord by the alpha₁ adrenoceptor.
agonist methoxamine and the short-duration rhythm produced by electrical stimulation of the VMM.

Our previous work has shown that bath application of the \( \alpha_1 \)-adrenoceptor agonist methoxamine produced an alternating left-right rhythmic pattern in sacral and in flexor dominated lumbar segments of the spinal cord (Gabbay and Lev-Tov 2004). Figure 5A shows ventral root recordings from the left and right S2 (LS2 and RS2) after addition of 100 \( \mu \)M methoxamine to the experimental bath. It can be seen that the rhythm produced under these conditions is volatile with rapid changes in the frequency and amplitude of bursting. Because of these rapid changes, Fourier-based analysis, even with short segments, cannot accurately capture the dynamic changes in behavior. However, such data can be readily analyzed using cross-wavelet and coherence analysis to characterize the left-right coupling in S2 during the rhythm. The coherence spectrum of the activity between LS2-RS2 is shown Fig. 5B. Two rhythmic components are evident: a faster high-coherence component characterized by a constant-phase alternating left-right pattern and by a gradual decrease in frequency with time (region 1), and a slower component distinguished by a constant-frequency and by time varying coherence and left-right phase-shift (region 2). The time course of changes in frequency of the two components during the rhythm is shown in Fig. 5C, and the variations in the mean coherence of the two components are shown in Fig. 5D. Circular plots of the phase and mean \( r \) vectors obtained for three consecutive data samples within the two regions of interest are shown in Fig. 5E. The

![FIG. 5. Wavelet analysis of nonstationary signals produced by bath-applied methoxamine. A: ventral root recordings (50 Hz to 10 kHz) from the left (L) and right (R) S2 segment of the spinal cord in the presence of 100 \( \mu \)M methoxamine. B: coherence spectrum of the interrelations between the left and right activity during the rhythm. Black arrows denote the mean phase values (clockwise, 180° pointing left). One and 2, regions of interest selected for analysis. C: variations in the frequency of the methoxamine induced rhythm. Data were divided into 4-s bins grouped into 5-point bins, to calculate the means and SD of regions 1 (brown) and 2 (green). D: variations in the mean coherence values within the regions of interest (regions 1, brown, and 2 green). Calculations of mean \( \pm \) SDs as in C. E: circular plots of mean phase and \( r \) vectors of 3 consecutive 80-s data segments. Time marks denote the segment midpoint. Region 1, 80 s: \( \phi = 180.73 \pm 33^\circ \), \( r_v = 0.85 \), \( n = 48,000 \); 160 s: \( \phi = 190.9 \pm 22.7^\circ \), \( r_v = 0.92 \), \( n = 48,000 \); 240 s: \( \phi = 185.2 \pm 26.2^\circ \), \( r_v = 0.9 \), \( n = 42,000 \). Region 2, 80 s: \( \phi = 186.13 \pm 15.56^\circ \), \( r_v = 0.96 \), \( n = 38,000 \); 160 s: \( \phi = 167.6 \pm 30.7^\circ \), \( r_v = 0.87 \), \( n = 42,000 \); 240 s: \( \phi = 122.75 \pm 15.76^\circ \), \( r_v = 0.96 \), \( n = 37,000 \).]
analyses described in the preceding text, emphasize the value of obtaining the dynamic portrait of the data and the misleading results that can be obtained by ignoring time-varying processes during the activity of the network.

The slow hidden component of the rhythm revealed by the WT analysis is consistent with the APV-sensitive slow descending noradrenergic modulation of the lumbar rhythm described in Gabbay and Lev-Tov (2004).

The final example in this work demonstrates the use of XWT and CWT in studies of the longitudinal coupling between the lumbar and sacral segments of the spinal cord during rhythmic activity produced by stimulation of the brain stem. In contrast to the drug-induced activity, tonic brain stem stimulation results in a brief (~20–80 s) episode of bursting in the lumbar and sacral cords. These episodes are too short and too nonstationary to be analyzed using Fourier-based methods but can be quantified with wavelet/coherence analysis.

Recordings were obtained (Fig. 6A) from the left (L) and right (R) L2 and S2 ventral roots, before (control), after a bilateral cut of the ventral funiculi at the lumbar-sacral junction (bilateral VF cut), and after a bilateral VF cut followed by a left ventrolateral funiculus cut (bilateral VF, L-VLF cut). These lesions change the coupling between the lumbar and sacral cords, which can be quantified by examining the coherence between the left lumbar and sacral segments. The coherence spectra of the interdependence between the left lumbar and sacral segments (LL2-LS2) are shown together with the respective rectified signals in Fig. 6B. The two regions of interest analyzed in this experiment after software-removal of the stimulus artifacts, reveal a slow (~0.6 Hz) high-coherence
frequency band developing during the train (control, B1, and D) corresponding to the burst frequency, and a faster (4 Hz) discontinuous frequency band with lower coherence (control B2 and D). Examination of the wide-band and the low-pass filtered signals revealed time-locked electrotonic potentials and firing produced during the 4-Hz stimulus trains. The strong lumbosacral coupling of the rhythmic bursts during the stimulus train (region 1; coherence = 0.93 ± 0.014 throughout the train) decreases to 0.82 ± 0.012 at the beginning of the stimulus train after the bilateral VF cut (Fig. 6, B and D) and then increases gradually with time (slope = 0.008, $P < 0.001$) toward the end of the train. The mean L2–S2 phase shift ($\varphi$, Fig. 6E) changes from 356 ± 13.6°, $rv = 0.972$, $n = 30,000$ in the control series to 24.3 ± 14.3°, $rv = 0.97$, $n = 30,000$ after the bilateral VF cut, while keeping substantial regularity throughout the respective trains. The L2–S2 coupling, however, does not reach a significant coherence and disappears when the bilateral VF cut was followed by a left ventrolateral funiculus (L-VLF) lesion (Fig. 6, B and D, blocked). On the other hand, the 4-Hz component (region 2), persists with a constant phase shift after the bilateral VF and the combined bilateral VF and L-VLF cut, at least throughout parts of the stimulus trains (Fig. 6E, control, 28 ± 26°, $rv = 0.902$, $n = 30,000$; bilateral VF cut = 29.5 ± 22.4°, $rv = 0.926$, $n = 27,500$; bilateral VF, L-VLF cut = 50 ± 22°, $rv = 0.93$, $n = 23,700$), suggesting that the descending fibers mediating this time locked component do not travel exclusively in the lesioned pathways.

DISCUSSION

In the present work, we used spectral statistical methods to analyze rhythmic patterns produced by activation of central pattern generators in the in vitro isolated preparation of the mammalian spinal cord. The analyses yielded measures of the regularity and frequency of the rhythm and of the interrelations between pairs of time series.

Our study showed that the use of WT with a Gaussian modulated sine wave, the Morlet function, yielded a satisfactory time-frequency localization of the signals produced by the central pattern generating circuitry. We propose the Morlet-based WT as the tool of choice for quantitative analyses of stationary and nonstationary rhythmic patterns induced either by neurochemical means or electrical stimulation of the brain stem or afferent pathways in the spinal cord. The advantages of fully automated computer analysis of oscillating networks are self-evident. The most frequently used methods for such analysis are based on Fourier transform of stationary neural signals. Most of the data examined in our study exhibited various degrees of nonstationarity so that the Fourier-based analysis had a rather limited value. This was demonstrated in Fig. 1, in which the ~0.3-Hz NMDA/5HT/DA-induced rhythm was modulated by slow oscillations. To allow Fourier-based analyses of these signals, data had to be divided into multiple windows to reach a semi-stationary state. However, when this was done, the windowed data were too short for reliable Fourier-based coherence tests (Fig. 2D) (e.g., Carter 1987; Wang et al. 2004). As a result, the coherence of the slow component of the rhythm did not reach statistical significance, and even the coherence of the faster component (corresponding to the burst frequency) was statistically significant only in part of the nonoverlapping windows (Fig. 2D). The situation becomes more complicated when the rhythmic patterns are induced by electrical stimulation of the brain stem (see Fig. 6) or of afferent pathways. In such cases, the Fourier-based coherence tests cannot be applied reliably because the duration of the rhythm is limited to tens of seconds by the inability of the stimulated pathways to maintain adequate activation for longer periods of time and because the evoked rhythm exhibits substantial variations with time. Thus the WT is one of few ways to perform rigorous statistical analysis of these electrically induced rhythms.

INTERPRETATION OF THE WT/COHERENCE. The routine analyses performed in this study included the WT spectra of each time series, the cross-WT of pairs of time-series variables, and the coherence spectra. The significance of the WT and the coherence spectra was examined using Monte Carlo simulations of series of white noise. The interpretation of the significant parts of the spectra is not always straightforward. First, inadequate use of the Monte Carlo procedure (such as inadequate number of iterations) may omit spectral regions with functional significance, and second, spectral regions that are highly significant do not necessarily have a physiological meaning (e.g., second harmonics). Attempts to remove small spectral regions that occupy less than a predetermined percentage of the matrix by smoothing in the time/frequency domain or applying thresholding techniques is also problematic because the filtered out regions may be meaningful. For example, the time-locked signals produced by stimulation of the brain stem appear as repetitive coherent spots at 4 Hz in Fig. 6 (region 2). Smoothing in the time/frequency domain or applying area-wise significance test of the coherence spectra (Maraun et al. 2007) filtered out most of the 4-Hz spots that were found to be significant using our point-wise significance test.

For these reasons, it is important to examine carefully both the significant WT and WT coherence spectra with respect to the relevant parts of the analyzed signals. Application of this strategy accompanied by assessment of the reproducibility of the results in series of experiments can assure the exclusion of higher harmonics of the signals and irrelevant time/frequency regions from the analyzed spectra.

STATISTICAL INFERENCE OF THE RHYTHMIC PARAMETERS. Several characterizing parameters can be extracted from the relevant regions of interest of the WT, XWT, and CWT spectra. These include the mean frequency and power of significant regions of the auto-WT, the mean shared frequencies and phase from the coherent cross-power spectra, and the mean coherence from the significant coherence spectra. Appropriate consecutive sampling throughout the time domain provides a reliable expression of variations of these parameters with time (see Figs. 3, 5, and 6). Further statistical inference including multiple comparisons of means and slopes of changes can be obtained by conventional methods of linear statistics. The phase data can be readily analyzed using circular statistics procedures for directionality and multiple comparisons (Zar 1999) (see Figs. 4–6). One should remember however that the XWT spectra from which the phase data were extracted are
actually matrices of complex numbers where the phase and power are integrated. There are ways to assess the relative contribution of the phase and power to the interrelations between time-series variables (Li et al. 2007). Although these analyses were performed in the present study, we preferred not to use them at this stage and rather rely on the integrated phase-power values. Another point that should be mentioned in this regard is the fact that the XWT and CWT analyses examine the linear relations between time-series variables in time/frequency domain. Nonlinear interactions between different frequency bands of the auto WT or between pairs of variables can be evaluated by wavelet bicoherence analysis (Jaech and Powers 1998; Stylili et al. 2007; van Millingen et al. 1995) as has been explicitly described in a recent study (Li et al. 2007). The results of bicoherence analyses of our data are beyond the scope of this work and will be reported in the near future.

In summary, the present work introduces the WT and WT coherence as tools for automated quantitative analysis of the nonstationary rhythmic patterns produced by the spinal pattern-generating circuitry. The analyses enabled us to characterize the dynamic profile of the signals, to assess the linear relation between spectra of any given pair of signals, and to uncover hidden components of the rhythm in the time/frequency domain. The quantitative indices extracted from the spectra can be used for routine assessment of motor rhythms in experimental animals and for assessing clinical treatments in spinal cord injury patients (A. Etlin, B. Shamir, G. Zeilig, and A. Lev-Tov unpublished studies).

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