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Use of Wavelet and Fast Fourier Transforms in Pharmacodynamics

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ABSTRACT

Progress has been made in the development and application of mechanism-based pharmacodynamic models for describing the drug-specific and physiological factors influencing the time course of responses to the diverse actions of drugs. However, the biological variability in biosignals and the complexity of pharmacological systems often complicate or preclude the direct application of traditional structural and nonstructural models. Mathematical transforms may be used to provide measures of drug effects, identify structural and temporal patterns, and visualize multidimensional data from analyses of biomedical signals and images. Fast Fourier transform (FFT) and wavelet analyses are two methodologies that have proven to be useful in this context. FFT converts a signal from the time domain to the frequency domain, whereas wavelet transforms colocalize in both domains and may be utilized effectively for nonstationary signals. Nonstationary drug effects are common but have not been well analyzed and characterized by other methods. In this review, we discuss specific applications of these transforms in pharmacodynamics and their potential role in ascertaining the dynamics of spatiotemporal properties of complex pharmacological systems.

Quantitative pharmacology involves the characterization of drug effects within and across scales of organization by means of integrating drug-specific properties and those reflective of physiological processes and control systems. A major goal is to identify specific factors that influence the time course of pharmacological effects; however, biosignals emanating from complex physiological systems often exhibit temporal variability. As opposed to simply errors in measurement assay, time-series analysis has revealed that such highly variable data are a product of nonlinear dynamical systems that can be described by chaos theory (Tallarida, 1990; van Rossum and de Bie, 1991; Dokoumetzidis et al., 2001; Goldberger et al., 2002). Traditional pharmacodynamic models are unable to recognize such complex and variable information.

High-frequency measurement of drug effects allows development of concepts of pharmacodynamic systems analysis and drug effects based on integrated signaling networks. There are many challenges to this approach; however, recognition of the basic tenets of the complexity, robustness, emergent properties, and intrinsic noise of biological signaling networks provide insight for their analysis (Weng et al., 1999; Aderem, 2005). Current approaches attempting to characterize such interactions in mechanistic terms include deterministic systems such as ordinary differential equations (e.g., chemical kinetics and compartmental models) and partial differential equations (e.g., reaction-diffusion models), stochastic systems (frequently used for species existing in small numbers), and hybrid systems that combine deterministic and stochastic components (Eungdamrong and Iyengar, 2004).

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ABBREVIATIONS: FFT, fast Fourier transform; CWT, continuous wavelet transform; DWT, discrete wavelet transform; EEG, electroencephalogram; 1-D, 2-D, 3-D, and 4-D, one-, two-, three-, and four-dimensional, respectively; STFT, short-time Fourier transform; FFHP, first Fourier harmonic projection; FRET, fluorescence energy resonance transfer; HRV, heart rate variability; HF, high-frequency; PET, positron emission tomography.

Mathematical transforms are required to interpret time-series data of nonlinear systems and instances in which the frequency content of a signal is more informative than the original waveform. Fourier transforms of various biomedical signals have identified primary frequency regions that reflect the interplay between physiological control systems, the understanding of which may be exploited to characterize the intensity and time course of in vivo drug effects. Wavelet transforms are an extension of Fourier-type spectral analysis; however, they localize in both time and frequency domains and have properties that may have utility for analyzing pharmacodynamic data. It is important to recognize that mathematical transforms do not represent techniques that are mutually exclusive from current modeling paradigms (i.e., deterministic and stochastic systems). Wavelet and Fourier transforms may be used to describe specific functions within these models, characterize biological system properties that are subject to modeling, explore interconnections of system components, and/or qualify properties of existing models. Here we provide a brief overview of the fundamentals of Fourier and wavelet analyses, present example applications in pharmacodynamic research, and discuss their potential for analysis of complex pharmacological systems.

The Fast Fourier Transform

Fourier transforms break down time-domain signals, $h(t)$, into constituent sinusoids of different frequencies, $H(f)$ (Brigham, 1988; Press et al., 1992):

$$H(f) = \int_{-\infty}^{\infty} h(t)e^{2\pi ift}dt \quad (1)$$

where H is amplitude, f is frequency (Hz), and $i = \sqrt{-1}$. The inverse Fourier transform is defined as:

$$h(t) = \int_{-\infty}^{\infty} H(f)e^{-2\pi ift}df \quad (2)$$

This shifts the time domain to a frequency domain. This transform also may be applied to space and position signals (e.g., imaging data), with H as a function of inverse wavelength (cycles per distance measure). For periodic functions, the original waveform may be reconstructed from the sinusoidal components by application of the Fourier transform:

$$h(t) = a_0/2 + \sum_{n=0}^{\infty} [a_n \cos(2\pi nt/T) + b_n \sin(2\pi nt/T)] \quad (3)$$

where n refers to the number of harmonics, a_i and b_i are the Fourier coefficients for the corresponding harmonics, and T is the period or time length of the waveform.

A useful feature of the Fourier transform (Brigham, 1988; Press et al., 1992) is to correlate two functions (g and h), defined as:

$$Corr(g, h) \equiv \int_{-\infty}^{\infty} g(\tau + t)h(\tau)d\tau \quad (4)$$

with the temporal shift τ leading to a Fourier transform pair:

$$Corr(g, h) \Leftrightarrow G(f)H^*(f) \quad (5)$$

where the Fourier transform of the correlation is the product of the transform of one function, $G(f)$, and the complex conjugate (*) of the transform of the other function. A variant is the correlation of a function with itself (autocorrelation), in which eq. 5 becomes:

$$Corr(g, g) \Leftrightarrow |G(f)|^2 \quad (6)$$

A second useful feature of the Fourier transform is the computation of total power (TP) within a signal:

$$TP \equiv \int_{-\infty}^{\infty} |h(t)|^2dt = \int_{-\infty}^{\infty} |H(f)|^2df \quad (7)$$

with $|H(f)|^2$ as the energy density function over frequency called the power spectrum or power spectral density. Power spectrum may also be defined as the Fourier transform of the autocorrelation function (eq. 6). The power within a frequency interval may be of interest and is calculated by the integral of $|H(f)|^2$ (eq. 7) over the relevant frequency range.

A discrete Fourier transform is often used for signals composed of data sampled at evenly spaced intervals. A continuous signal may be reconstructed without information loss if the sampling frequency (f_s) is greater than twice the highest frequency component in the signal (Nyquist critical frequency) (Semmlow, 2004). The discrete Fourier transform is defined by the equation:

$$H_n \equiv \sum_{k=0}^{N-1} h_k e^{2\pi ikn/N} \quad (8)$$

with N as the total number of data points. The FFT is used to compute the discrete Fourier transform and power spectrum because it reduces the number of computations from N^2 to 2 log N. The power spectral density of a signal composed of two sinusoidal frequencies is shown in Fig. 1, A and B.

Physiological waveform data generally represents truncated data segments of continuous signals [e.g., electroencephalogram (EEG) and electrocardiogram]. Direct application of the FFT is typically modified by windowing and averaging to reduce artifacts in the power spectrum resulting from the analysis of truncated data. The FFT-computed power spectrum is an approximation of the true spectrum, and averaging may be used to improve its statistical properties.

FFT and Spectral Analysis in Pharmacodynamics

Biorhythmic Physiological Baseline Functions

Physiological control systems, such as hormone secretion, cardiovascular signaling and function, temperature regulation, and metabolism, often exhibit circadian rhythms; however, their integration is not well defined. Fourier analysis

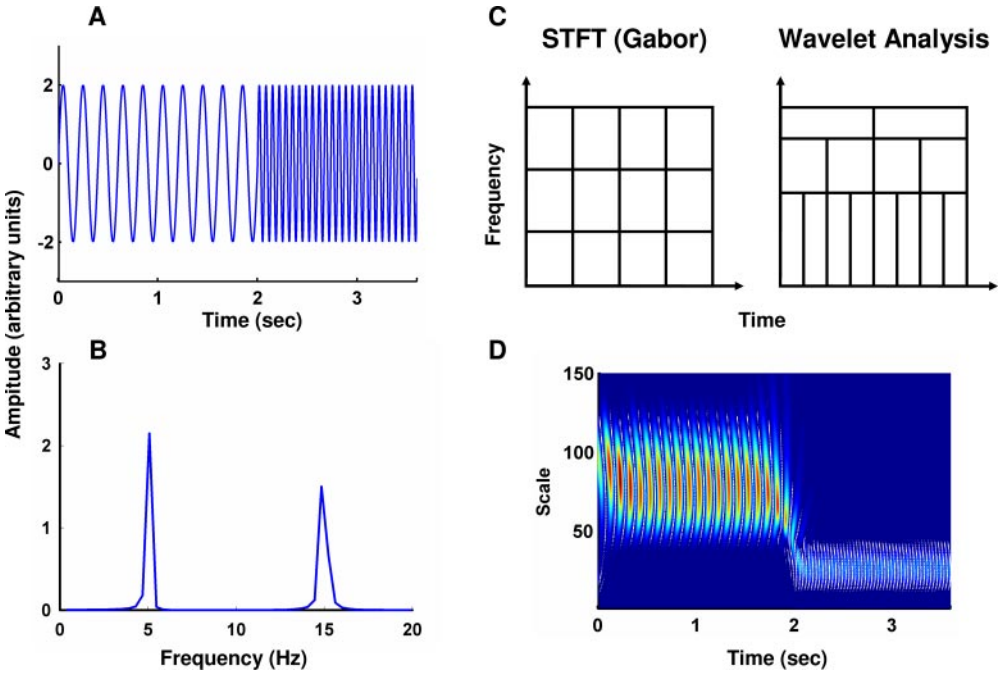

Fig. 1. Examples of traditional spectral and wavelet analysis. A, simulated signal containing two sinusoids of equal amplitude at frequencies of 15 Hz for the first 2 s and 5 Hz for the times thereafter. B, power spectrum of the signal using the FFT and a rectangular window (periodogram). C, schematic comparing the windowing aspects of the STFT and wavelet transform. The STFT is associated with a window of fixed length, which compromises either frequency or temporal resolution, whereas the wavelet transform utilizes variable windows attempting to capture high frequencies with shorter windows and low-frequency content with longer segments. D, color map of squared wavelet coefficients (power) of the signal as a function of scale (inverse frequency) and time. Scales 23 and 70 correspond with frequencies of 15.3 and 5 Hz according to eq. 23.

can be used to characterize the time course of such periodic signals in response to drug treatment.

To model circadian cortisol concentrations, Krzyzanski et al. (2000) developed an algorithm that uses Fourier series analysis to derive periodic, time-dependent input functions for basic turnover pharmacodynamic models that have been coded as a Fortran executable program called FOURPHARM. The rate of change of a biological substance (R) undergoing periodic secretion can be described by the following equation:

$$dR/dt = K_{in}(t) - k_{out} \times R \quad (9)$$

with $K_{in}(t)$ as a time-dependent zero-order production rate and k_{out} as a first-order elimination rate constant. A square L^2-norm approximation algorithm was established to determine a minimal number of harmonics (N) and an approximate (but explicit) function that characterizes the circadian baseline profile (eq. 3). The Fourier coefficients can be used to derive an approximate solution to $K_{in}(t)$, such that:

$$K_{in}(t) = A_0 + \sum_{n=1}^{N} [A_n \cos(2\pi nt/T) + B_n \sin(2\pi nt/T)] \quad (10)$$

where,

$$A_0 = a_0 k_{out}/2 \quad (11)$$

$$A_n = a_n k_{out} + b_n 2\pi n/T \quad (12)$$

$$B_n = b_n k_{out} - a_n 2\pi n/T \quad (13)$$

With the input function defined, the baseline equation (eq. 9) can be integrated into indirect pharmacodynamic response models, where drug concentrations may serve to inhibit or stimulate the production or loss of the response variable. For cortisol, the pharmacokinetics of exogenous corticosteroids serve as driving functions for inhibiting $K_{in}(t)$. This is achieved by multiplying $K_{in}(t)$ in eq. 9 by the following inhibition function:

$$I(C) = 1 - \frac{I_{max} \times C_p}{IC_{50} + C_p} \quad (14)$$

where C_p is the plasma concentration of exogenous steroid, I_{max} is the maximal inhibition factor ($0 < I_{max} \leq 1$), and IC_{50} is the plasma steroid concentration producing 50% I_{max}. Model parameters (k_{out}, I_{max}, and IC_{50}) are estimated from fitting eqs. 9 to 14 to temporal cortisol profiles using nonlinear regression analysis. The Fourier coefficients may be fixed to values determined using the L^2-norm approximation algorithm or, alternatively, could be estimated. This method improves on previous techniques for modeling circadian cortisol dynamics and has been extended to jointly model the combined effects of cortisol and exogenous corticosteroids on lymphocyte trafficking (Mager et al., 2003). Recently, this approach was used to describe the circadian rhythm of endogenous corticosterone that was used as a driving function to characterize the periodic profiles of glucocorticoid receptor and glutamine synthetase expression (Yao et al., 2006). The general Fourier approach outlined by eqs. 9 to 13 may be applied to other nonstationary biological baselines and modified to include additional mechanisms of drug-induced effects.

Markers of Pharmacological Effect from Spectral Analysis

Akselrod et al. (1981) were the first to show that parasympathetic, sympathetic β-adrenergic, and renin-angiotensin system blockade alters specific components of the power spectrum of beat-to-beat heart rate fluctuations. Frequency-domain based methods for computing the power spectral density of heart rate variability (HRV) data include the FFT and parametric autoregressive modeling (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The primary frequency bands are high-

frequency (HF) HRV (0.15–0.4 Hz) that reflects autonomic parasympathetic tone and corresponds to the change in heart rate that occurs with breathing and the low-frequency HRV region (0.04–0.15 Hz) that represents a complex measure of sympathetic and vagal nerve activity. Analysis of heart rate variability has provided insight into sympathetic and parasympathetic tone in response to genetic background and various physiological, pharmacological, and pathological perturbations in humans (Pagani et al., 1986; Malliani et al., 1991; Craft and Schwartz, 1995; Liao et al., 1995; Thayer et al., 2003; Welzig et al., 2003).

The paradoxical effect of atropine on parasympathetic cardiovascular control, where low doses of the drug cause an increase in vagal tone (shown as an increase in the HF band of the power spectrum) and high doses can result in complete parasympathetic blockade (Alcalay et al., 1992), was demonstrated using FFT analysis. However, frequency-domain techniques are limited by loss of temporal information (Fig. 1B). One method for exploring the time course of frequency content of signals is to divide the signal into shorter segments of equal length and determine the frequency band power in each segment from the FFT (termed short-time Fourier transform or STFT) (Fig. 1C). Parametric techniques, such as autoregressive modeling, are an alternative to using the FFT to calculate power spectra and may also be used with this segmenting approach (Semmlow, 2004). The time epochs must be of sufficient duration to identify the frequency range of interest but short enough to allow good temporal resolution. Scheinin et al. (1999) applied this window technique to monitor the parasympatholytic effects of atropine, glycopyrrolate, and scopolamine. The parasympathetic tone in short segments of approximately 5 min was defined by the Hayano index (*HI*) (Hayano et al., 1990):

$$HI = \frac{\sqrt{HFP}}{RR} \times 100 \quad (15)$$

where *HFP* is the high frequency power from autoregressive modeling of the power spectrum and *RR* represents the mean interbeat interval. To accommodate hysteresis in the concentration-effect curves, an effect compartment pharmacodynamic model was used for atropine and glycopyrrolate; however, a weakness of this method, *HFP* noise, precluded analysis of the scopolamine concentration-effect profile.

To address the intrinsic noise of HRV spectra using a cumulative plot approach, peak values of the power spectrum in the HF range for male Sabra rats (1.35–2.65 Hz) were obtained from autoregressive modeling of 2-min epochs of interbeat intervals following intravenous injection of saline, scopolamine, or a range of atropine doses (Perlstein and Hoffmann, 2000). Although these HF peak values fluctuate significantly over time, a plot of the cumulative HF peak values as a function of time under placebo conditions reveals a constant positive slope, suggesting stationary vagal tone. Cumulative plots after low-dose scopolamine and atropine show an increase in this slope, whereas high atropine doses produce less steep curves, both gradually returning to predose values as drugs are eliminated. Thus, in this instance, the cumulative plot of spectral measures provided a relatively simple and rapid means of monitoring the time course of pharmacological effects of drugs on the autonomic nervous system. The parasympatholytic effects of atropine were then modeled using an indirect response model [eqs. 9 and 12 with $K_{in}(t)$ replaced by a constant zero-order rate constant defined as $K_{in} = k_{out} \times R(0)$], with the slope of the cumulative plot representing the pharmacodynamic response variable (Perlstein et al., 2001). Hoffman and co-workers also developed a model to characterize the increase in vagal tone induced by low doses of scopolamine and atropine (Perlstein et al., 2002), where a significant delay in the onset of effects required the use of a time-dependent transduction model (Mager and Jusko, 2001).

Similar short-time frequency analysis methods have been applied to EEG signals for quantifying the pharmacodynamics of drugs for the central nervous system, including benzodiazepines, neuroactive steroids, and other $GABA_A$ receptor modulators, antipsychotics, synthetic opiates, and adenosine A_1 agonists (Mandema and Danhof, 1992; Danhof, 2002). An example of mechanism-based modeling of spectral measures of central nervous system drug effects utilized measurement of the average amplitudes in the β-frequency band (11.5–30 Hz) of 1-min EEG signals. These signals, obtained using FFT, were measured in male Wistar rats following 5-min intravenous infusions of one of nine study drugs, including six benzodiazepines, zolpidem, zopiclone, and a β-carboline compound (Visser et al., 2003). The final pharmacodynamic model represents a modified form of the operational models of agonism described by Black and Leff (1983), where the ultimate effect is defined by the two distinct processes of drug-receptor interaction and subsequent signal transduction. The estimated in vivo pharmacodynamic parameters were well correlated with those obtained from in vitro bioassays.

Functional Image Analysis

The study of intracellular pharmacodynamics has been hampered by a lack of understanding relationships between biochemical modulation and direct drug effects, localized cellular genomic and protein expression patterns, and the spatiotemporal kinetics of reactants and cofactors. In addition, as opposed to traditional pharmacodynamic modeling, low concentration of chemical species at this subcellular level of organization may require the use of stochastic dynamics (Holcman and Schuss, 2005; Siegel and Ramanathan, 2005). For these systems, cellular and subcellular molecular imaging may provide a suitable framework for characterization of drug action. Fluorescence resonance energy transfer (FRET) may be used to determine the proximity of labeled molecules for monitoring protein-protein interactions (Kenworthy, 2001), as well as conformation changes in functional signaling reporters (Kunkel et al., 2005). To quantify and study the dynamics of such cellular and subcellular processes, the Fourier transform may be extended to two dimensions and applied to images or spatial signals to determine the spectral content of images. Issues related to sampling theory as described for time-series data also apply to spatial signals, and the analogous spatial frequencies are given as cycles per unit length or sample. The two-dimensional Fourier transform is an extension of eq. 1 and is defined as:

$$H(f_1, f_2) = \int_{m=-\infty}^{\infty} \int_{n=-\infty}^{\infty} h(m, n) \times e(2\pi i f_1 m) \times e(2\pi i f_2 n) dm dn \quad (16)$$

where f_i terms are the spatial frequencies and m and n are spatial coordinates in an x-y plane. Thus, the corresponding two-dimensional discrete Fourier transform for an M by N image is:

$$H(n_1, n_2) \equiv \sum_{k_1=0}^{M-1} \sum_{k_2=0}^{N-1} h(k_1, k_2) \times e(2\pi i k_1 n_1/M) \times e(2\pi i k_2 n_2/N) \quad (17)$$

As with the one-dimensional discrete Fourier transform, the FFT can be used to efficiently compute the transform coefficients of $h(k_1,k_2)$.

Use of FFT to describe cellular dynamics may be approached by extending principles of fluorescence correlation spectroscopy. Petersen et al. (1993) have developed image correlation spectroscopy for the quantitative analysis of images generated from confocal scanning laser microscopy. Consider a one-dimensional autocorrelation function (eq. 4) specified in the spatial domain:

$$Corr(g, g) \equiv \int_{-\infty}^{\infty} g(\xi + x)g(x)dx \quad (18)$$

where ξ represents a spatial shift. An important property of this function is that the variance of the fluctuations in the original signal can be defined by eq. 18 as $\xi \rightarrow 0$, which is inversely proportional to the number of fluorescent particles. Analogous to eq. 6, the two-dimensional FFT can be used to calculate a 2-D autocorrelation function. A 2-D Gaussian function is fitted to the normalized autocorrelation function using standard nonlinear regression analysis, and the number and density of fluorescent particles are calculated from model parameter estimates. Changes in the aggregation state and distribution of receptors for platelet-derived growth factor on human foreskin fibroblasts were detected using this approach (Petersen et al., 1993; Wiseman and Petersen, 1999). Recently, Wiseman et al. (2004) extended this methodology to include the time-domain (time-lapse sequences) to map the spatiotemporal dynamics of α5-integrin in migrating cells.

Visualization of Multi-Dimensional Datasets

Systems analysis in pharmacodynamics requires large datasets of exquisitely measured multiple response outputs. Zhang et al. (2003, 2004) have introduced a Fourier transform based method for representing and analyzing multidimensional datasets. Mapping of multidimensional data into a 2-D plot is achieved by identifying the first harmonic of the Fourier projection. The FFT is applied to N-dimensional data, and the resulting 2-D coordinates are specified by the real and imaginary components of the first harmonic of the discrete Fourier transform (eq. 8) that can be plotted in both Cartesian and polar plots. This is the first Fourier harmonic projection (FFHP) and is applied in the software program VizStruct (online implementation available at http://www.cse.buffalo.edu/DBGROUP/bioinformatics/supplementary/vizstruct). In addition to pharmacogenomic examples, application of VizStruct to experimental and simulated pharmacokinetic data allows demonstration of small differences in individual subject pharmacokinetic parameters (e.g., total systemic clearance), as well as data simulated using different structural models (standard 1- and 2-compartment models constrained to identical net exposures following single intravenous doses). The FFHP method may be relatively insensitive to noise in the data, making it a potentially useful tool for pharmacological data analysis.

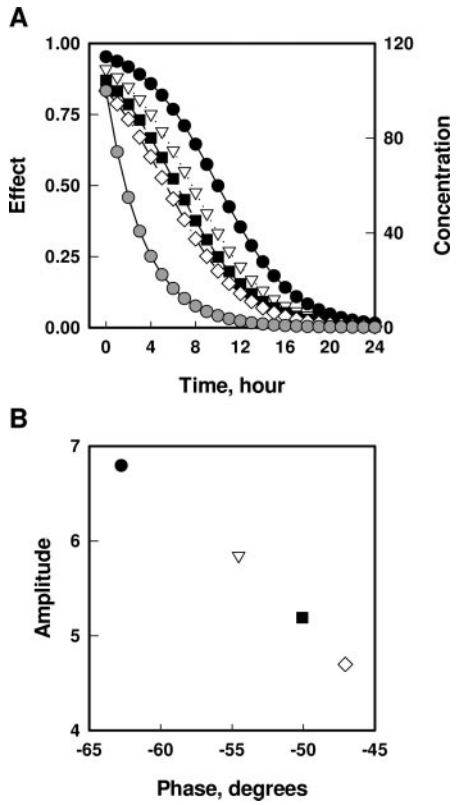

Fig. 2. FFHP of simulated pharmacodynamic data. A, time course of simulated concentrations (gray circles) and subsequent response profiles using the Hill function (eq. 19), with EC_{50} values of 5 (●), 10 (▽), 15(■), and 20 U (◇). B, FFHP of the pharmacodynamic time series where the x- and y-axes are the real and imaginary components of the first harmonic calculated using the FFT.

An application of the FFHP to pharmacodynamic data are shown in Fig. 2. Four pharmacodynamic profiles were simulated using the Hill function:

$$E = \frac{E_{max} \times C}{EC_{50} + C} \quad (19)$$

corresponding to four different EC_{50} values (5, 10, 15, and 20 U), where $E_{max} = 1$ and a single monoexponential function for concentration (C) was used as the driving function: $C = C_0 \times e^{-k \times t}$ (C_0 and k fixed to 100 U and 0.3 h^{-1}). Similar to the pharmacokinetic examples, the FFHP can identify small differences in model parameters, in this case drug sensitivity, used to generate the pharmacodynamic time-series (Fig. 2B). Additional examples include the use of the FFT in global sensitivity analyses for model identification and, more interestingly, the classification of multiple response variables that could be used to explore mechanistic relationships between system components (Gueorguieva et al., 2005).

Continuous Wavelet Transform

A major assumption of frequency-domain techniques for spectral analysis is that signal structures are stationary over

time, a condition that is rarely observed for physiological systems. Although the power spectral density describes the power distribution over frequency, the time at which changes in power and/or frequency occur in these signals cannot be determined. Gabor (1946) introduced the concept of the STFT, and as discussed previously, this adaptation maps a signal into a function of time and frequency (see Fig. 1C):

$$STFT(f, t) = \int_{-\infty}^{\infty} h(\tau)[w(t-\tau)e^{2\pi i f\tau}]d\tau \quad (20)$$

where $w(t - \tau)$ represents the sliding window function. However, only limited time resolution is obtained using this technique that is dependent on the size of the fixed window used to analyze the signal and the density of the measurements. The STFT is recognized as providing either good time or frequency resolution but not both (Wiklund et al., 1997).

The continuous wavelet transform (CWT) represents a natural extension of Fourier analysis and provides a windowing technique of variable-sized regions based on frequency (see Fig. 1C). Similar to the FFT, the wavelet transform denotes a shift in data representation from the time domain of the original signal to an alternate domain. The CWT is defined as:

$$W(a, b) = \frac{1}{\sqrt{a}}\int_{-\infty}^{\infty} h(t)\psi^*\left(\frac{t-b}{a}\right)dt \quad (21)$$

where a represents scale (inversely related to frequency), b is the translation parameter (shift in time or space), and ψ is a wavelet basis function. Thus, scale or frequency information is obtained, whereas the function simultaneously localizes in the time domain. Similar to Fourier analysis, where a signal is decomposed into a series of *sin* and *cos* functions, the CWT is the sum over all time of a signal multiplied by scaled (a) and shifted (b) versions of the chosen wavelet basis function. The calculated coefficients, $W(a,b)$, reflect the correlation between the original signal, $h(t)$, and the wavelet basis function at specific scales (or frequencies) as a function of time. There are several families of wavelets, and although each has specific features that might enable certain applications, the final choice of the basis function is often made experimentally. The Morlet wavelet is an example of those defined by explicit functions over all time:

$$\psi(t) = e(-t^2)\cos(\pi\sqrt{2/\ln 2} \times t) \quad (22)$$

In contrast, the Daubechies (db) wavelets are the limits of an iterative process and equal zero outside a specific interval or region of support (Daubechies, 1992). This so-called compact support, in addition to other properties, makes the db wavelets well suited for local signal analysis.

Wavelet analysis of a simulated signal exhibiting a change in frequency content at a specific point in time is shown in Fig. 1D. The signal represents a simple sinusoid with a frequency of 15 Hz for the first 2 s and 5 Hz for times thereafter (Fig. 1A). The power spectrum of this signal calculated with the FFT shows no indication of the temporal aspects of the signal. The square of the wavelet coefficients as a function of scale and time is shown in Fig. 1D. For point of reference, scales 23 and 70 correspond to frequencies of 15.3 and 5.0 Hz, as described by the relationship:

$$f_a = \frac{f_c}{a \times \Delta} \quad (23)$$

where f_c is the center frequency of the wavelet and Δ is the sampling period. The time course and change in frequency content of the simulated signal are clearly detected using wavelet analysis (Fig. 1D).

Wavelet Analysis in Pharmacodynamics

Biosignal Analysis in the Time-Frequency Domain

The need for techniques that simultaneously assess time and frequency information of biomedical signals in analyzing cardiovascular function is well known (Novak et al., 1997; Mainardi et al., 2002). Wavelet analysis of cardiac interbeat interval has been used to monitor cardiovascular control systems in various disease states and with therapeutic interventions (Ivanov et al., 1996; Joho et al., 1999; Toledo et al., 2003).

Parametric Wavelet Transforms in Medical Imaging

The multiresolution properties of the CWT and its time/space-frequency colocalization make it well suited for biomedical image analysis (Laine, 2000). One relatively direct application is the use of wavelet transforms to de-noise images for subsequent analysis. Millet et al. (2000) used a 1-D discrete wavelet transform (DWT) to design a filter for [^{11}C]flumazenil PET scans. The DWT is often represented by its inverse, such that any function [$h(t)$] may be defined as (Semmlow, 2004):

$$h(t) = \sum_{j=-\infty}^{\infty}\sum_{k=-\infty}^{\infty} c_{j,k}2^{-j/2}\psi_{j,k}(2^{-j}t - k) \quad (24)$$

where the coefficients ($c_{j,k}$) are evaluated at discrete points j and k, such that the dyadic scale and translation (a and b in eq. 21) are defined as 2^j and 2^jk, respectively. Data from the [^{11}C]flumazenil PET scans had spatial resolution of the wavelet-based filter that was superior to traditional filtering techniques, including the Fourier-based methods. Filtered PET signals representing concentration-time profiles were subsequently fitted using a parametric mechanism-based pharmacokinetic model. Ligand-receptor binding was included in the compartmental model, which permitted the estimation of in vivo target density and binding parameters from noninvasive image analysis in humans.

Turkheimer et al. (2000) developed algorithms for characterizing the time-varying spatial pattern of radioactivity in PET studies that include multiresolution analysis and linear modeling in the wavelet domain. The overall procedure involves: 1) the application of the DWT to each 2-D image in a time-lapse sequence, 2) kinetic modeling of the time course of each wavelet coefficient to obtain a parametric wavelet transform and associated standard error, 3) utilization of the parametric wavelet transform to threshold the coefficients, and 4) application of the inverse DWT to the remaining coefficients to obtain the final spatial mapping. This technique may be applied to 4-D data sets (3-D images in time) and has been extended to provide variance estimates of the spatial mapping from an analysis of the residuals of the kinetic modeling

in the wavelet domain (Aston et al., 2005). Applications of the DWT for analyzing magnetic resonance imaging studies have also been reviewed previously (Bullmore et al., 2004). Whitcher et al. (2005) propose to group voxels according to a wavelet-based clustering algorithm. This technique involves applying the DWT to each voxel time series within user-selected regions. A subset of coefficients is selected as a means of dimension reduction, and members of the final set are grouped by *k*-means clustering. The algorithm was successfully applied to several data sets, including phantom data, contrast-enhanced perfusion-weighted imaging, and the pharmacokinetic analysis of an i.v. contrast agent injected into rats.

Identification of Cellular Signaling Microdomains

A 4-D description of drug and protein-protein subcellular interactions, where both space and time components are simultaneously considered, would be of considerable interest (Bork and Serrano, 2005). Kobrinsky et al. (2005) described a wavelet-based approach to identifying signaling microdomains in cell membranes (Fig. 3A). The CWT was applied to FRET microscopic images of COS-1 cells expressing a membrane localized genetically encoded reporter of protein kinase C activity. Stimulation of COS-1 cells with either phorbol-12,13-dibutyrate or acetylcholine provided 2-D signals of FRET originating from the cellular membrane that were subjected to linearization, with 1-D signals generated at several time points before and after exposure to the agonists (Fig. 3B). The wavelet transform was applied to each 1-D signal, and differences in wavelet coefficient matrices, compared with control images (Fig. 3C), were used to initially identify potential microdomains. Transient activity in several domains was heterogeneous and would not have been identified by evaluating average activity over the entire membrane. Such techniques will permit exploration of local heterogeneity in cell signaling.

Potential Role in Integrative Pharmacology

Wavelet-based methods of analysis may have considerable use to interpret both data originating from and mathematical models describing complex pharmacological systems. For example, Sosnovtseva et al. (2005a,b) developed a double-wavelet approach for studying the dynamics of cellular and physiological systems. Instantaneous frequencies and amplitudes of signals determined using the wavelet transform are used as input signals for a second wavelet analysis. This approach serves to identify major frequencies, as well as potential modulators, associated with specific cellular and physiological processes. With dense experimental data, wavelet analysis may also be used as a form of mathematical model identification and/or validation. Gorbunova and Spitzer (2002) compared wavelet coefficient matrices of transformed signals of transient Ca^{2+} spikes induced by cyclic AMP in spinal neurons (*Xenopus* embryos) with those simulated using a theoretical model. This could be used to confirm that the model recapitulates the dynamics of the frequency content as a function of time and thus the potential mechanisms influencing the system.

In summary, the fast Fourier and wavelet transforms are widely used for the analysis of complex nonlinear dynamical systems in bioengineering and many other fields of study. Many signals of physiological and pharmacological processes

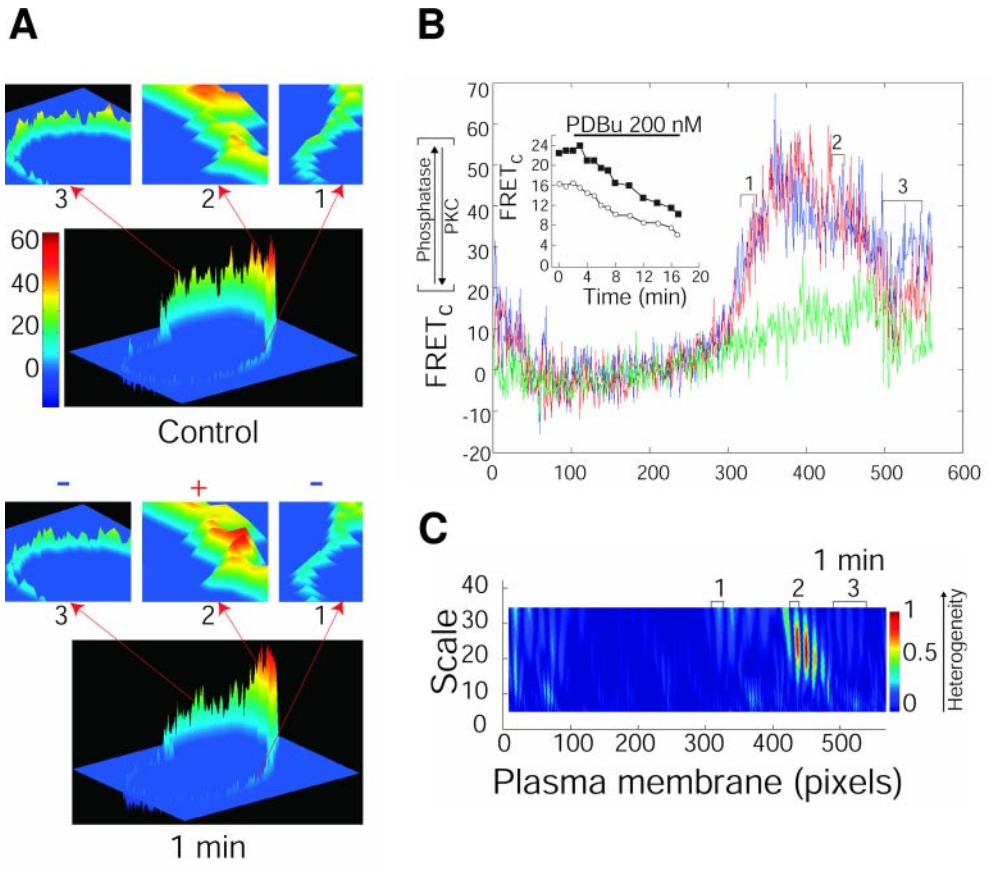

Fig. 3. Signaling microdomain identification in COS-1 cell plasma membrane. A, three-dimensional representation of protein kinase C activity before (control) and 1 min after treatment with phorbol-12,13-dibutyrate (PDBu) expressed as corrected values of fluorescence resonance energy transfer ($FRET_c$). Arrows point to three microdomains as defined using the wavelet-based algorithm described in text. The increase in intensity after 1 min in region 2 (positive sign) is in agreement with the average membrane intensity (B, inset), whereas regions 1 and 3 show decreased intensity (negative sign). B, $FRET_c$ values in the linearized plasma membrane before (blue) and 1 (red) and 15 min (green) after PDBu treatment. The numbered regions correspond with those in A. C, difference between the wavelet coefficient matrix obtained before and 1 min after PDBu treatment. Color map represents normalized square differences and numbered regions correspond with those in A and B. Although only the 1-min plot is shown, difference matrices are used to identify potential domains that are verified using a statistical test of the variance in $FRET_c$ values within each region over time. Figure adapted from Kobrinsky et al. (2005) with permission from the Biophysical Society.

exhibit such properties, where significant information may be contained in the time-frequency domain as opposed to the original waveform. Although the FFT and other spectral methods have been integrated into traditional methods of pharmacodynamic systems analysis, the use of wavelet transforms is more preliminary. Further development of these mathematical transforms may better incorporate time and space parameters into pharmacodynamic analysis.

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