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Journal of Neuroscience Methods 99 (2000) 137–141

JOURNAL OF NEUROSCIENCE METHODS

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Utility of the wavelet transform to analyze the stationarity of single ionic channel recordings

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Received 4 January 2000; received in revised form 19 April 2000; accepted 19 April 2000

Abstract

Wavelet transform, a time-scale analysis, is presented as a new tool to analyze single-channel recordings. This method makes it possible to verify the stationarity, to identify episodes of change in the kinetic channel behavior (burst, flickering, cooperativity) or episodes of noise, and to localize stationary segments in long single-channel current recordings. It can help the conventional analysis of the kinetic behavior of ionic channels leading to better understand the gating mechanism.

Keywords: Wavelet transform; Ionic channel; Patch-clamp; Signal processing

1. Introduction

It is now thought that ionic channels, an extremely large group of heterogeneous proteins having the ability to form a pore for the passive movement of ions across membranes, are present in most cell types and play an important role of signal transduction in the cellular physiology. An electrophysiological technique, the patch-clamp (Hamill et al., 1981), has sufficient resolution to record currents through individual ionic channels of biological membranes. Ionic channels control the passive flux of selected ions by gating their pores in response to various factors such as agonists or membrane potential. The gating is thought to arise from a series of conformational changes. Channels open and close very quickly. Thus, the currents through channels appear to be rectangular current pulses of random duration but fixed amplitude if the electrochemical gradient does not change. The stochastic behavior of single-channel current in a steady state has been interpreted as the channel's state transitions between several open and shut states, and these transitions have been regarded as a homogeneous Markov process with a definite transition rate constant at each transition step (Colquhoun and Hawkes, 1995). Kinetic analysis of single-channel current is important to understand the gating mechanism of an ion channel; it requires the acquisition of sufficiently long records of stationary single-channel currents.

A stochastic process is called stationary if the probability of being in each state does not depend on time. Without sudden change introduced by the experimenter during the recording, the important sources of nonstationarity for nonvoltage-dependent channels are an unintentional drift in the experimental conditions, a run-down of the preparation or possible bursting and flickering activities of channels. These changes can constitute an important artefact in the kinetic analysis of long records of single-channel currents. Thus, it is very important to verify the stationarity of these recordings. With this aim, we present here a new tool, the wavelet transform (WT), which expands a signal into a time-scale representation, similar to a time–frequency representation. WT has already been used in the signal processing of biomedical signals (Akay, 1995) and even, in our laboratory, for the spike detection in electroencephalogram signals (Clochon et al., 1993; Clarençon et al., 1996) but never in patch-clamp data analysis. In this latter domain, we show that the wavelet analysis is

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0165-0270/00/$ - see front matter
PII: S0165-0270(00)00230-2

a performing tool to verify the hypothesis of stationarity, and that it can detect and localize stationary segments in long single-channel current recordings.

2. Material and methods

2.1. Introduction to wavelet transform

As proposed by Morlet et al. (1982) for seismic signal analysis, the continuous wavelet transform $C_g(a,b)$ of a signal $s(t)$ is the decomposition of this signal onto a set of basis functions $g_{a,b}(t)$:

$$C_g(a,b) = \langle s, g_{a,b} \rangle = \int_{-\infty}^{\infty} g^*_{a,b}(t)\, s(t)\, \mathrm{d}t \tag{1}$$

where $g^*_{a,b}(t)$ is the complex conjugate of $g_{a,b}(t)$.

The basis functions $g_{a,b}(t)$ are obtained from a given analyzing wavelet $g(t)$ by dilatations or contractions (time-scale parameter a) and by time shifts (parameter b):

$$g_{a,b}(t) = \left(\frac{1}{\sqrt{a}}\right) g\left(\frac{t-b}{a}\right) \tag{2}$$

Computing the WT of a signal consists of mapping the signal into a time-scale plane. The notion of scale (parameter a) is introduced as an alternative to frequency. Wavelet function $g(t)$ is often a time complex function, as the function defined by Morlet et al. (1982):

$$g(t) = \pi^{-1/4}\, \mathrm{e}^{-\mathrm{i}kt}\, \mathrm{e}^{-t^2/2} \tag{3}$$

Therefore, WT is a complex-valued function and it conveys both modulus and phase information. Both are necessary to reconstruct the signal. However, a description based only on the squared modulus, providing an energy density distribution and referred to as scalogram throughout this paper, is often preferred. The scalogram distributes the energy E_g of the signal all over the time-scale plane (Rioul and Flandrin, 1992):

$$\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} |C_g(a,b)|^2 \frac{\mathrm{d}a\, \mathrm{d}b}{a^2} = E_g \tag{4}$$

The real time function, the so-called 'Mexican hat' function (Daubechies, 1989), is also currently adopted for the wavelet function $g(t)$:

$$g(t) = \frac{2}{\sqrt{3}} \pi^{-1/4} (1 - t^2)\, \mathrm{e}^{-t^2/2} \tag{5}$$

This wavelet function presents one of the best possible simultaneous concentration properties both in time and frequency domains. The corresponding piecewise constant function, the so-called 'top hat' wavelet of Eq. (6), is currently adopted for an approximation of the 'Mexican hat' function (Arneodo et al., 1989).

$$g(t) = \begin{matrix} 1 & \text{if } |t| < 1 \\ -0.5 & \text{if } 1 < |t| < 3 \\ 0 & \text{if } |t| > 3 \end{matrix} \tag{6}$$

We have chosen this latter wavelet because its shape was close to the rectangular current pulses of single-channel recording. Since this function is a real function, the wavelet transform is a real-valued function.

2.2. Computing wavelet transform

The discrete wavelet transform has been obtained by computing the convolution of the digitized signal with this latter analyzing wavelet after discretization of a and b parameters. A software program has been written in our laboratory with Labwindows/CVI (National Instruments, USA). It runs on a PC microcomputer under a Windows 3.1x or higher environment. It opens data files in ASCII format and in Biopatch 3.41 (Bio-Logic software, France) format. Modulus, squared modulus, and eventual phase of WT can be calculated for the wavelet functions of Eqs. (3), (5) and (6). After defining the required parameters as a choice of analyzing frequencies, computing is applied to all points of the entire data file. Results are saved on the hard disk before displaying. They can be displayed in time-scale representation in false colors (thermal color palette) or in inverse grey scale (luminance palette), and in a time graph for each frequency. The threshold and maximum of WT can be defined for the application of linear or logarithmic color or grey scale. Time-scale representations and time graphs can be filtered. Moving or zooming into the signal is possible. In the case of condensed representations, displayed results represent the arithmetical mean of WT round the point, the number of data for computing each mean value being in proportion to the condensation. In the case of extended representations, linear interpolation is applied between calculated WT for displaying additional pixels.

3. Results

To demonstrate the nature of the WT produced, two test signals were shown.

First, Fig. 1 shows a time-scale representation of the energy density distribution based on the squared modulus of WT (scalogram) of an ionic channel recording where we have introduced a noisy segment made by linear combination with a sinusoidal function at 50 Hz frequency. A 'top hat' wavelet is used. The ability of the transform to localize in both time and scale this noisy segment is clearly evident.

Second, we wished to demonstrate the utility of the wavelet analysis in the detection of breaking-down points of the stationarity in the kinetic behavior of

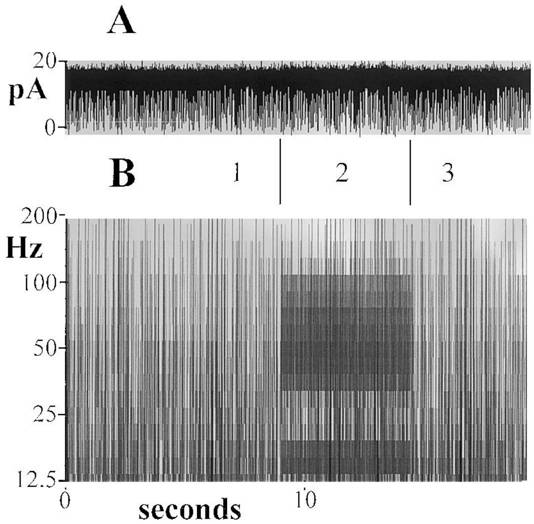

Fig. 1. Wavelet transform of a noisy ionic channel recording obtained using the ‘top hat’ wavelet. (A) Signal was formed by a single-channel recording filtered at 3 kHz and digitized at 9600 Hz, where the second part was a linear combination with a sinusoidal function (frequency, 50 Hz; amplitude, 1 pA). (B) Time-scale representation of energy density distribution based on the squared modulus of wavelet transform (scalogram). Seventeen frequencies are shown in the vertical axis, distributed according to three sub-octaves between 12.5 and 200 Hz. The inverse grey scale is applied after a logarithmic conversion of WT values. A dark color represents a high-energy density.

ionic channels, and in the localization of stationary segments into signals. We chose to show the analysis of an artificial signal created by concatenation of stationary segments. The stationarity of these segments was obtained by computing, with Biopatch 3.41 software, simulated ionic channel currents according to the theory of Markov chains. A white gaussian noise was artificially created with Biopatch 3.41 software, and mixed with the signal in order to reproduce the aspect of a biological signal. Fig. 2A represents a signal formed by concatenation of four segments of 1 min simulating stationary kinetic behavior of two ionic channels. The parameters of kinetic models for the simulation and the signal-to-noise ratio were chosen so as to make difficult the visual detection of stationary segments into the signal. Fig. 2B,C, respectively, represent the time-scale scalogram and the time plots of squared modulus of WT for three analyzing frequencies selected among the 13 calculated. As shown in these figures, this easy signal processing makes possible, in one stage and in a blind way, the localization of the four stationary segments. Therefore, a precise cutting out of the signal in stationary segments makes possible the analysis of the kinetic behavior of ionic channels by using Biopatch 3.41 software. So, for each cutting segment, after verifying the functional independence of the two channels and computing the dwell time distributions in the three observable current levels corre-

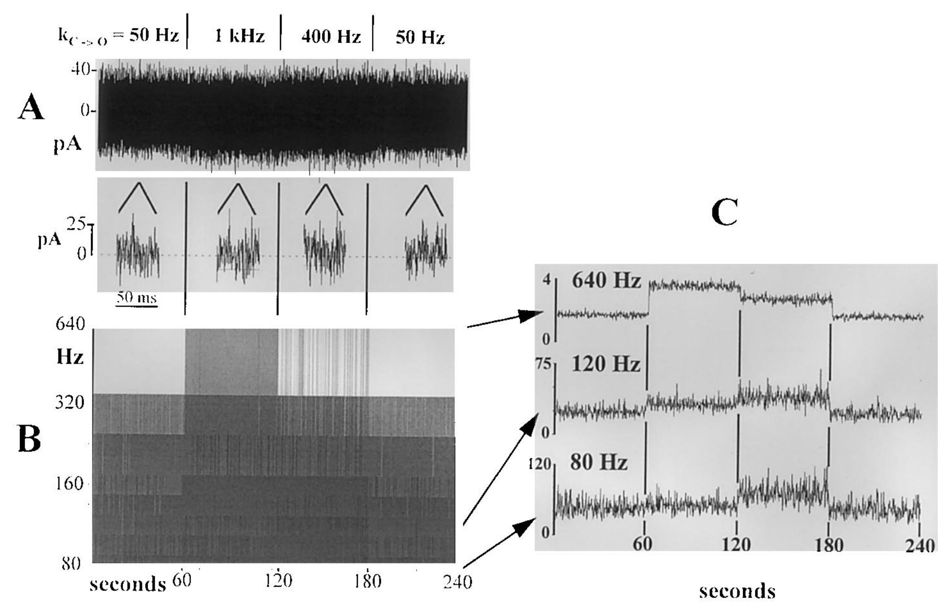

Fig. 2. Wavelet transform of a simulated single-channel recording obtained using the ‘top hat’ wavelet. (A) Signal formed by concatenation of four stationary segments of 1 min. The kinetic model for the simulation of each stationary segment is formed by two ionic channels with, for each channel, one closed state, C, and one open state, O. The unitary current is 10 pA. The kinetic constant $k_{O\to C}$ is fixed at 400 Hz for the four simulations, and $k_{C\to O}$ is successively 50, 1000, 400 and 50 Hz. An offset is applied to reset the mean of each stationary segment. A simulated white gaussian noise is added (mean, 0; standard deviation, 6.66 pA). The signal-to-noise ratio defined as the ratio of unitary current to standard deviation of noise is deliberately chosen low, equal to 1.5. The sampling frequency (SF) is 3 kHz. The simulation software rules out oscillations of frequencies greater than SF/2. No additional filtering process is performed. (B) Scalogram using the ‘top hat’ wavelet function. Thirteen frequencies are distributed according to three sub-octaves between 80 and 640 Hz. Inverse grey scale is applied after a logarithmic conversion of WT values. (C) Time plots of squared modulus of WT for three analyzing frequencies: 640, 120 and 80 Hz (arbitrary scale in ordinate).

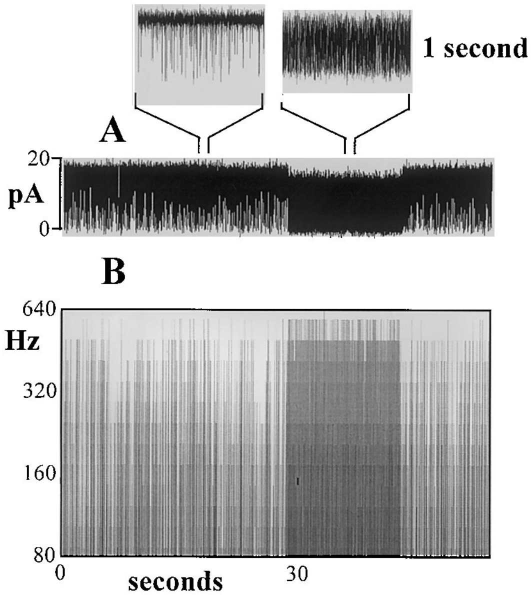

Fig. 3. Wavelet transform of BK(Ca) channel recording. (A) Single-channel current recording lasting 54.6 s in inside-out configuration on N1E-115 cells. (B) Scalogram using the 'top hat' wavelet function. Thirteen frequencies are distributed according to three sub-octaves between 80 and 640 Hz. Inverse grey scale is applied after a logarithmic conversion of WT values.

sponding to the three open states (0, 1 and 2 open channels), the two time constants of the kinetic model calculated with the use of conventional methods (Colquhoun and Sigworth, 1995) are close to the initial values that are used for the simulations. The power spectral density separately calculated from each cutting segment shows that the powers released in the same frequency bands as those studied for WT follow a comparative evolution as the squared modulus of WT from one segment to another. This observation strengthens the idea that the scalogram is a time-scale representation of energy density distribution.

Therefore, WT can be used to verify the stationarity, to identify episodes of change in the kinetic channel behavior and to localize stationarity segments in long single-channel current recordings without important filtering. Fig. 3 shows the scalogram of an ionic channel recording filtered at 3 kHz and digitized at 9600 Hz. The signal represents the current through one large conductance calcium-activated potassium channel BK(Ca) on N1E-115 neuroblastoma cells. This channel, which we have previously characterized (Diserbo et al., 1994, 1996), is here recorded in inside-out configuration under symmetrical [K^+] (145 mM) and pCa 5.3 in the bath, and clamped to a membrane potential of 60 mV. No change was introduced by the experimenter during the recording but an apparent change in the kinetic behavior of this channel occurred. As shown in Fig. 3B, a scalogram using the 'top hat' wavelet is proved as a good tool to detect in a blind way this episode. During this episode, the energy density appears higher and shifts to higher frequencies. This pattern is representative of an episode of fast flicker process. Such a long flickering episode is rare in BK(Ca) channel recordings in N1E-115 cells, but is classically described in other cells (Yellen, 1984). In this example, we can distinguish three stationary segments according to the energy density distribution. There is no obvious difference between the patterns of energy density distribution of segments that precede and follow the flickering. This observation is confirmed by the classical analysis methods (amplitude distributions, dwell-time distributions in open and close states, autocovariances and power spectral densities) (data not shown).

4. Discussion and conclusion

One important goal in the analysis of the ion channel is to obtain a kinetic model for the gating. Kinetic models provide working hypothesis for studies relating structure to function. The kinetic analysis methods usually applied require the acquisition of long records of stationary single-channel currents. In a stochastic signal, such as the one met in a single-channel current recording in a steady state, stationarity is relevant to the time invariance of the probabilistic behavior. The mean value and the variance function are time invariant. Therefore, currently, methods to analyze stationarity of these signals consist of cutting signals in segments and of verifying that means and variances of current amplitude are constant all along the segmentation. Another method is the analysis of variance in a variance–mean plot. These methods are dependent on the cutting out of the record and cannot make it possible for a precise localization in time of the possible change in gating behavior. A stationary stochastic signal is also fully characterized by a unique time-independent spectral description, its power spectral density function. In nonstationary signals, the power spectral density appears not sufficient for a physically meaningful description. The time-scale analysis (like WT analysis) provides an alternative approach to analyze these nonstationary signals, and gives an approximation of the instantaneous energy for a given time and frequency. As presented in this paper, the wavelet transform in the scalogram mode appears as a good tool to verify the stationarity and to localize stationary segments in long single-channel current recordings. It can be used without important filtering, i.e. without important distortion of the signal, and it gives information about the energy distribution in the time–frequency plane. It therefore acts as a 'mathematical microscope' through which one can observe different parts of the signal by adjusting the focus. In Fig. 1, we have also demonstrated the utility of WT in elimination of noisy signal segments. Other applications of the WT can be tested in the domain of patch-clamp signal processing. For example, in BK(Ca) channel currents recorded in a cell-attached configuration on excitable cells, WT can help to detect perturbations or rhythmic activity produced by calcium waves in cytosol or action potential in the kinetic behavior of these channels. WT can also be useful for detecting changes in cooperativity between the channel complex since power spectral density function is known to be a very accurate indicator of cooperativity (McGeoch and McGeoch, 1994). WT can also help to study bursting in channel activity, or action of drugs and toxins on kinetic behavior of ionic channels. Therefore, WT may be considered as an efficient tool that complements the currently used tools in single-channel recording processing.

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