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Rescaled range analysis applied to the study delayed rectifier potassium channel kinetics

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Abstract

The gating of ion channels has widely been modeled by assuming that the transitions between open and closed states are a memoryless process. Nevertheless, analysis of records of unitary current events suggests that the kinetic process presents long lags (antipersistent correlation). Here, using the patch-voltage clamp technique and the rescaled range method, activity of single-channel delayed rectifier K^+ channels was studied. The experiment result showed that reversal potential was -73.3 mV in cell-attached mode. For the sequences of alternating open and shut time intervals, the Hurst coefficients were calculated for four different pipette potentials in rat dorsal root ganglion neurons. $H=0.34169\pm0.00672$ ($n=4$) for $V=-30$ mV; $H=0.34632\pm0.0142$ ($n=3$) for $V=-40$ mV; $H=0.39237\pm0.0113$ ($n=4$) for $V=-50$ mV; $H=0.3954\pm0.0012$ ($n=4$) for $V=-60$ mV. When the Hurst method was applied to the results from a simulated four-state Markovian model, it showed that it had different experimental data H coefficient, the distribution of the data values had no correlations between them, in particular, $H=0.2531\pm0.00403$ ($n=50$) for $V=-40$ mV. This indicates that open-dwell times and closed-dwell times are long lag (namely, antipersistent correlation) and do not change with the pipette potential applied to the patch.

Keywords: Rat dorsal root ganglion neurons; Delayed rectifier K^+ channels; Reversal potential; Patch-voltage clamp; Rescaled range method

1. Introduction

Ion channels are transmembrane proteins that form ion-selective pores in lipid bilayer membranes. These channels open and close spontaneously and may be observed using patch clamp recording [1]. The gating mechanism is of considerable interest because of the dominant role that channels play in the control of membrane potential and other key cellular processes [2]. Hodgkin and Huxley measured the electric current through the cell membrane of a giant nerve fiber clamped under different voltage and ionic conditions [3], and subsequently reformulated by Fitzhugh [4], modeling of ion channel gating has been rooted in the concepts of classical kinetics. In accord with these concepts, channel gating kinetics have been assumed to exist in a finite number of discrete

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states; the additional assumption that the transition rate constant among the states is independent both of time and of the previous channel activity defines the model as a time-homogeneous Markov chain model.

Although Markov chain models often fit experimental data with increasing numbers of states until the fit fails to improve [5,6], Libovitch et al. [7–9] applied Mandelbrot's (1983) fractal concepts to study the gating kinetic of ion channels. In contrast to Markov model assumptions, it is assumed that the channel can exist in an infinite number of energy states; the closed and open states are each represented as a continuum of many conformational states. The kinetic rate constant for leaving the closed or open states is then a mixture of the rate constants for leaving this collection of states. The fractal model proposed that this effective rate constant has the form At^{1-D}, where A is the kinetic setpoint, t is the time the channel has resided in the current state and D is the fractal dimension. This form was chosen because many other physical systems composed of processes that occur over a large range of spatial or temporal scales display this type of scaling.

A few techniques have been developed for analyzing fractal time series, among which is the rescaled range analysis [10]. The method is comparatively simple and rather faithful [11]. Hurst himself has found that for many natural phenomena H is, in average, approximately 0.73, that is, the phenomena exhibited correlation rather than random time series. Many biological processes were successfully examined for their fractal nature by different statistical measures, but the Hurst method was used only in a few cases. Recently in cellular biology, the rescaled range analysis has been used to analyze records in time produced by the mechanical motions of cells growing in tissue culture [12] and the R/S analysis was applied to the study of patch clamp records of human T-lymphocytes [13]. Ref. [15] showed that for the Ca^{2+}-activated K^+ channels of Leyding cells, the Hurst exponent was equal to 0.75, that is, the successive openings and closings were assumed to be a process with memory; similarly Refs. [14,16] reported rescaled range method applications.

We used the Hurst method to examine the data obtained on the single delayed rectifier potassium channel in rat dorsal root ganglion (DRG) neurons. The analysis was aimed at revealing open- and closed-dwell time antipersistent correlation. The results were compared with those obtained on simulated data for the model four-state Markovian model.

2. Method

2.1. Isolation of dorsal root ganglion neurons

Two- to three-week-old Sprague–Dawley rats, irrespective of sex, were decapitated, and the thoracic and lumbar segments of vertebrate column were dissected and longitudinally divided into two halves along the median lines on both dorsal and ventral sides. The rat DRG neurons together with dorsal and ventral roots and attached spinal nerves were taken out from the inner side of each half of the dissected vertebrate and transferred into Dulbecco's Modified Eagle's Medium (DMEM, Sigma) at pH 7.4. After the removal of attached nerves and surrounding connective tissues, the DRGs were minced with iridectomy scissors and incubated with enzymes including trysin (type III, Sigma) 0.5 mg/ml, collagenase (type IA, Sigma) 1.0 mg/ml and DNase (type IV, Sigma) 0.1 mg/ml in 5 ml DMEM at 35 °C in a shaking bath for 40 min. To stop the enzymatic digestion, 1.25 mg/ml soybean trypsin inhibitor (type II-S1, Sigma) was added. The isolated neurons were transferred into a 35-mm culture dish and kept still for at least 30 min. All experiments were performed at room temperature (20–30 °C) [17–20].

2.2. Solutions and electrophysiology

Single-channel recording was carried out at room temperature utilizing an EPC-9 patch clamp amplifier (Germany), using the cell-attached configuration and the inside-out configuration of the patch clamp method. Recording of single potassium channel current from DRG showed cell-attached bath solution contained (in mM) NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, TTX 0.001, $CdCl_2$ 0.2, D-glucose 10; inside-out config-

uration bath solution contained (in mM) NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, D-glucose 10; the patch-pipette (internal) solution contained NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, TTX 0.001, $CdCl_2$ 0.2. in external solution, its osmolarity was adjusted to 340 mOsm with sucrose and pH was adjusted to 7.4 with NaOH. The pipettes had resistances of 8–12 M Ω after backfilling with an internal solution.

Single-channel current recorded was used EPC-9 (Germany), current signals were filtered with a cutoff frequency of 1 kHz (eight-pole Bessel) and sampled at 20 kHz; for single-channel analyses, we used data from patches in which only one K^+ channel was present.

3. Theory

3.1. The R/S analysis

The rescaled range analysis or R/S Hurst analysis is used to study records in time or a series of observations in different time. Hurst spent a lifetime studying the Nile and the problems related to water storage. He invented a new statistical method, the rescaled range analysis (R/S analysis) [10]. The problem is to determine the design of an ideal reservoir on the basis of the given record of observed discharges from the lake. Here the method of Hurst will be introduced and considered as a time dependent function ξ_i; we divide it into $N(T)$ adjacent segments, each of T points. Performing the rescaled range analysis requires that we compute a quantity called R/S for each T. For eliminating possible trend influence, the mean of the nth segment of length T is first computed:

$$\langle\xi\rangle_{n,T}=\frac{1}{T}\sum_{i=(n-1)T+1}^{nT}\xi_i$$

The standard deviation $S_{n,T}$ of the nth segment of length T is defined as

$$S_{n,T}=\left[\left(\frac{1}{T}\right)\sum_{i=(n-1)T+1}^{nT}(\xi_i-\langle\xi\rangle_{n,T})^2\right]^{1/2}$$

For each point i in the time series, we compute

$$X_{n,T}(i)=\sum_{k=(n-1)T+1}^{i}(\xi(k)-\langle\xi\rangle_{n,T})$$

$$R_{n,T}=\max(X_{n,T}(i))-\min(X_{n,T}(i))$$

We computed the rescaled range $(R/S)_{n,T}$ of that segment

$$(R/S)_{n,T}=\frac{R_{n,T}}{S_{n,T}}$$

and averaged the rescaled ranges computed from the segments

$$(R/S)_T=\left(\frac{1}{N(T)}\right)\sum_{n=1}^{N(T)}(R/S)_{n,T}$$

where $N(T)=N_t/T$ and N_t was size of the samples.

We calculated the rescaled ranges for different duration T, and the logarithm of $(R/S)_T$ is plotted vs. the logarithm of T. The slope of this plot is H, the Hurst coefficient. When $0<H<0.5$, the self-similar correlations at all time scales are antipersistent, that is, increases at any one time are more likely to be followed by decreases over all later time scales. When $H=0.5$, the self-similar correlations are uncorrelated. When $0.5<H<1$, the self-similar correlations at all time scales are persistent, that is, increases at any one time are more likely to be followed by increases over all later time scales. H index was calculated with adjacent open and closed time intervals.

4. Results

The sealing of a glass micropipette onto the surface of a rat DRG neuron results in the appearance of unitary current events in approximately 75% of the attempts. Fig. 1 shows traces of single-channel currents recorded at different pipette potentials. As can be seen, the channels are voltage dependent: the probability of having an open channel (P_o) increases with depolarization.

The single-channel conductance was calculated from current amplitude and membrane potential correlation. Fig. 2 shows I–V plots; membrane

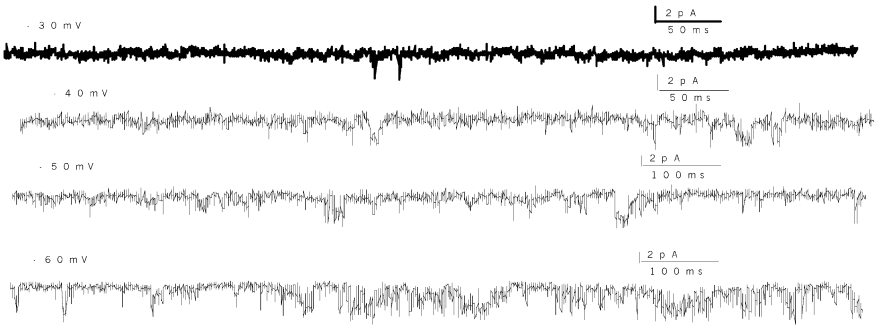


Fig. 1. Traces of single-channel currents recorded at different pipette potentials.

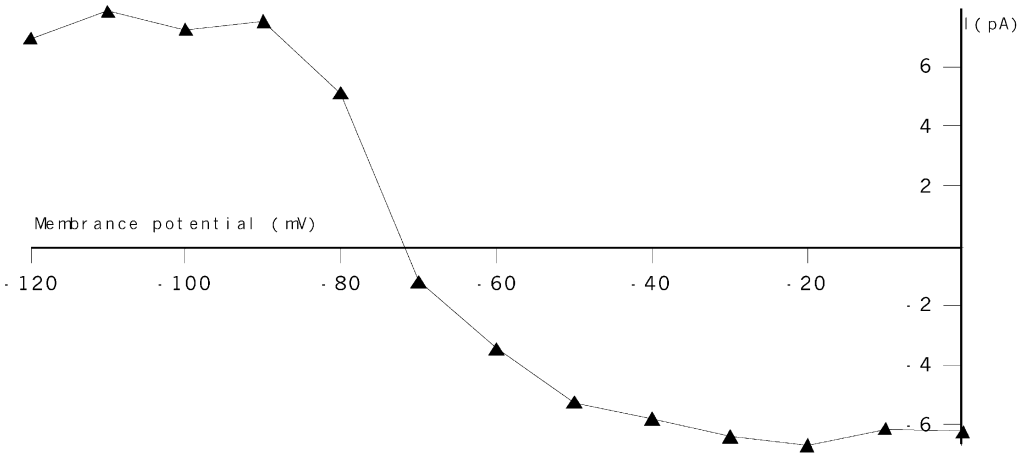


Fig. 2. *I–V* plots.

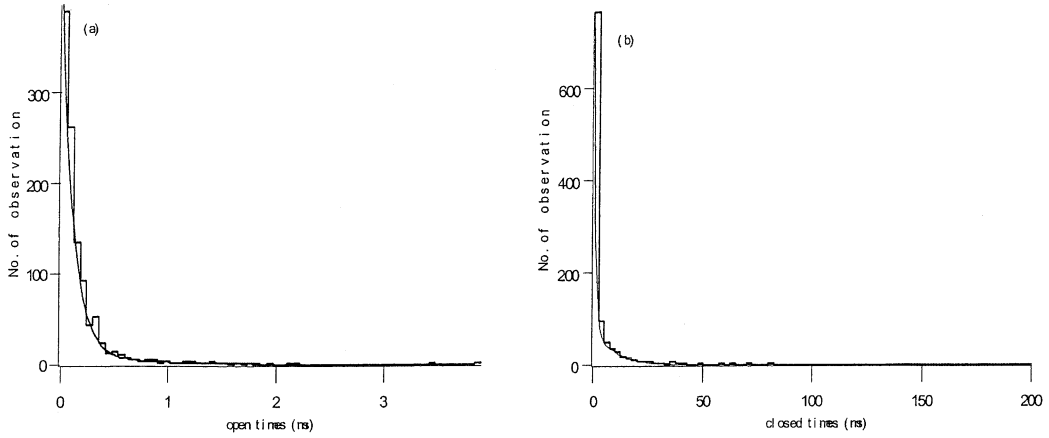


Fig. 3. Open and closed decay time histogram.

conductance was 20 pS, reversal potential approximately −73.3 mV. In cell-attach configuration, (EGTA 10 mmol/L) was presented both in the bath and inside the pipette. As calcium-activated potassium channel had large conductance as described [15]. Suggesting that we are dealing with a delayed rectifier K^+ channel.

Fig. 3 shows distributions of the residence times in the open (Fig. 3a) and closed states (Fig. 3b) for a pipette potential at −40 mV. As can be seen, the open time distribution can be adequately fitted by at least two exponentials for a good fitting, with decay time constants 0.12 and 0.83 ms. On the other hand, the closed time distribution also requires at least two exponentials for a good fitting. In this case, the decay time constants are 0.81 and 8.7 ms for the slow and fast components, respectively.

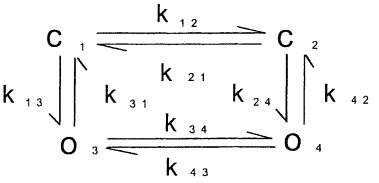
The p.d.f.s that describe the open and closed time distributions were fitted by the following exponential function: $f(t)_{\text{open}} = 0.1786e^{-1.2035t} + 7.1817e^{-8.43313t}$ and $f(t)_{\text{closed}} = 0.0652e^{-0.114617t} + 0.5296e^{-1.22767t}$ for pipette potential −40 mV.

A series double-logarithmic plot of the rescaled range R/S vs. the size of the samples (N_t) for typical experimental record of the delay rectifier potassium channel voltage clamped at −30, −40, −50 and −60 mV is shown in Fig. 4a, b, c and d, respectively. The Hurst coefficient was obtained from the slope of the line fitted through the experimental points.

Values of H calculated for different pipette potential applied through the patch are shown in Table 1.

Another interesting result is that the experimental values of H do not change with voltage, as shown by ANOVA at 5% significance level ($F = 2.251$; $P = 0.155$).

A four-state Markovian model was simulated using rate constant values so that the fractional open time was equal to the value of the fractional open time of the experimental data, according to the following kinetics scheme: Scheme 1. The given kinetic rate constants were as follows: $k_{12} = 250$, $k_{21} = 100$, $k_{13} = 1000$, $k_{31} = 6333$, $k_{24} = 10$, $k_{42} = 1000$, $k_{34} = 2000$, $k_{43} = 200$, for $V = -40$ mV. The results show $H = 0.2531 \pm 0.00403$ ($n = 50$) for $V = -40$ mV. Fig. 5 shows that R/S analysis



Scheme 1.

applied to simulated single-channel data. Fig. 5a and b shows the rate constant that was described in the text trace plot and double-logarithmic plots for $V = -40$ mV, respectively.

5. Discussion

The basic question involved in the study of time series resulting from a sequence of measurements of some quantity that fluctuates in time is to find a statistical model that best describes its properties. In our case, the base problem was to find out if there is long lag correlation between the open and closed time intervals without assuming that the kinetics of the channel follows a Markov process and Poisson process; this aim was achieved with the use of the rescaled range analysis (R/S analysis). The rescaled range analysis used here was $0 < H < 0.5$; this low value of H for different pipette potential records indicates a significant antipersistent correlation that arises from nonlinear interactions between different time correlated single-ion channels causing long lag time correlation in the macroscopic record of the membrane potential. The experimental results show that Hurst coefficient did not change with pipette potential. At the same time, the simulated results show that the randomized data have a different value of the Hurst exponent than the original data; although the H exponent is different from $1/2$, the data were randomized.

In this paper, we recorded that current of K^+ single channels is dependent on voltage in rat DRG neurons, using patch clamp; the conductance was 20 pS. At the same time, we also researched to distinguish between channel open-time and close-time, as well as its kinetics characteristics. In cell-attached mode, reversal potential was

−73.3 mV, which is near to K^+ balance voltage of used solution, which can prove that the channel we recorded is the K^+ single channel. In this experiment, both the pipette solution and the external solution have $CdCl_2$ 0.2 mmol/l and stop the Ca^{2+} from flowing into the cells. In this condition, we can also record K^+ currents; it was suggested that the channel was not calcium-activated potassium channel. *I–V* plot shows that the channel had

Table 1
Experimental values of *H*

Pipette potential (mV)	*H* (mean ± S.D.)	*N*
−30	0.34169 ± 0.00672	4
−40	0.34632 ± 0.0142	3
−50	0.39237 ± 0.0113	4
−60	0.3954 ± 0.0012	4

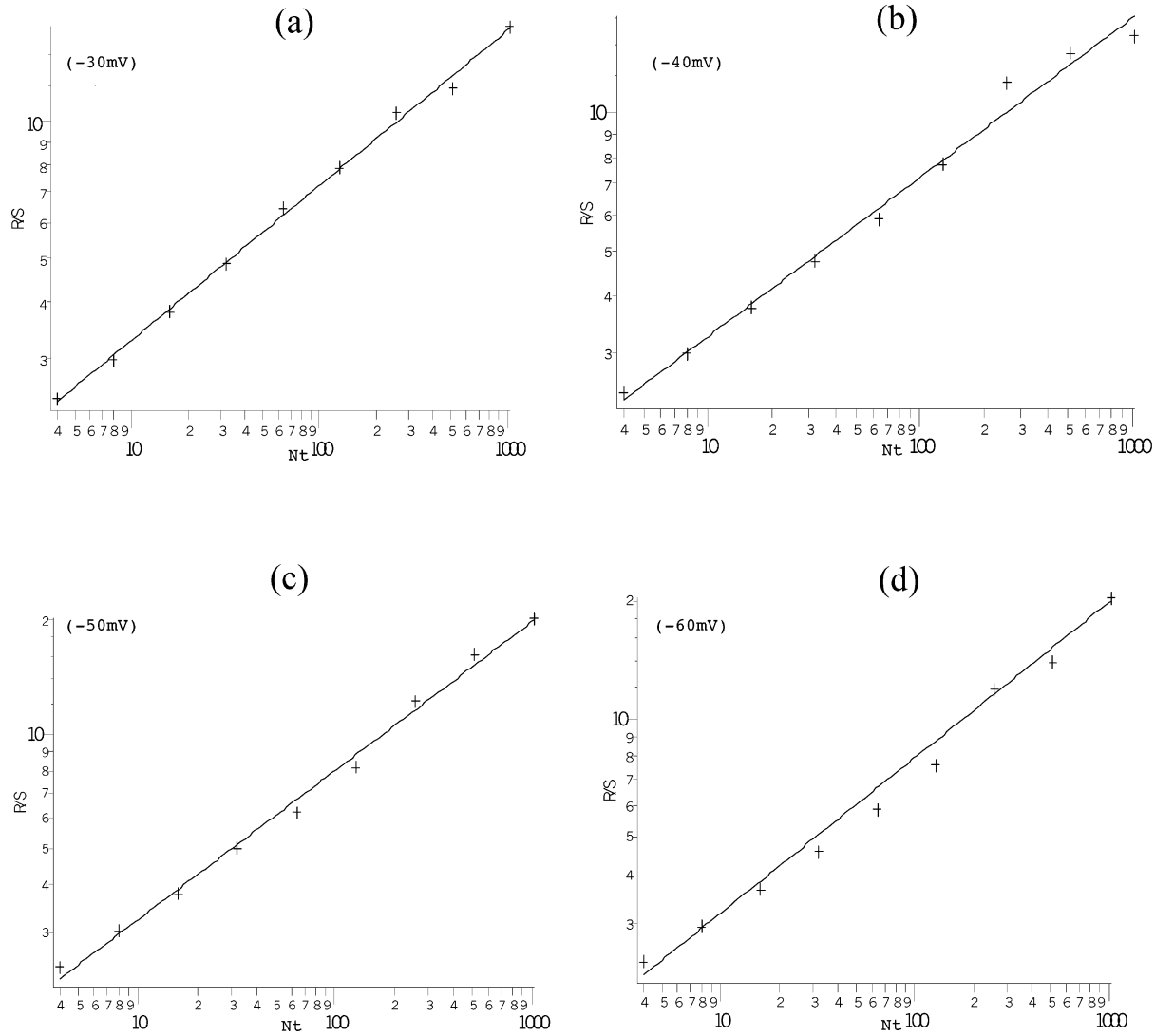


Fig. 4. A series double-logarithmic plot of the rescaled range R/S vs. the size of the samples (N_t) for typical experimental record of the delay rectifier potassium channel, pipette potential at −30, −40, −50 and −60 mV.

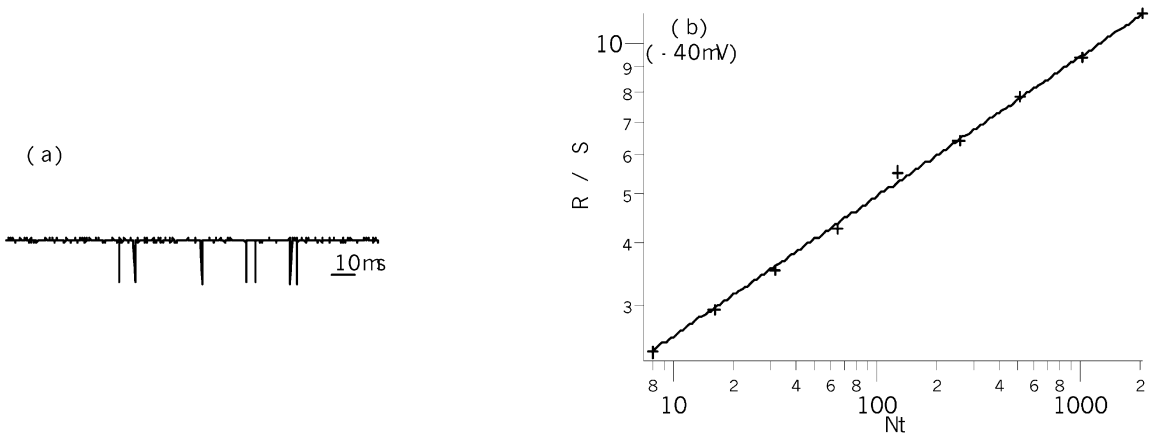


Fig. 5. (a) The rate constant that was described in the text trace plot and (b) R/S vs. N_t double-logarithmic plots for $V=-40$ mV.

delayed rectifier character, so it is suggested that this channel was not A current potassium channel.

The experimental results revealed that the conductance of K^+ channels was 20 pS, which was different from other neurons, such as pallium and hypothalamic, the conductances of which were approximately 50 pS. The conductance of hippocampal neurons was 15 pS–20 pS. Thus, it can be seen that delayed rectifier K^+ channels have different proteinic structures.

Finally, it is interesting to note that patch clamp data analysis of the distributions of the closed- and open-dwell times are insufficient to differentiate between Markov and fractal models. However, studies of the higher-order correlation properties of the data, for example the Hurst analysis carried out here, and molecular dynamics of the ion channel proteins and class different ion channel with *H* index should be the future direction of research.

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