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Correlation character of ionic current fluctuations: Analysis of ion current through a voltage-dependent potassium single channel

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Abstract

The gating of ion channels has widely been modeled by assuming the transition between open and closed states is a memoryless process. Nevertheless, the statistical analysis of an ionic current signal recorded from voltage dependence K^+ single channel is presented. Calculating the sample auto-correlation function of the ionic current based on the digitized signals, rather than the sequence of open and closed states duration time. The results provide evidence for the existence of memory. For different voltages, the ion channel current fluctuation has different correlation attributions. The correlations in data generated by simulation of two Markov models, on one hand, auto-correlation function of the ionic current shows a weaker memory, after a delayed period of time, the attribute of memory does not exist; on the other hand, the correlation depends on the number of states in the Markov model. For $V_p = -60$ mV pipette potential, spectral analysis of ion channel current was conducted, the result indicates that the spectrum is not a flat spectrum, the data set from ionic current fluctuations shows considerable variability with a broad 1/f -like spectrum, $\alpha = 1.261 \pm 0.24$. Thus the ion current fluctuations give information about the kinetics of the channel protein, the results suggest the correlation character of ion channel protein nonlinear kinetics regardless of whether the channel is in open or closed state.

Keywords: Ion channel; Correlation analysis; Memory; Single channel recording; Potassium channel; Dorsal root ganglion neurons

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 channels played 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 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. There is, however, clear evidence about the cases in which the behavior of ion channel current has been found to be non-Markovian, a method of testing Markovianity was proposed by Fulinski et al. [5]. They used a definition of the Markov process based on the Smoluchowski−Chapman−Kolmogorov equation. The methods applied to potassium current through a big conductance locust potassium channel (BK channel) revealed a non-Markovian character of channel action. Testing the Markov condition in ion channel recordings was proposed by J. Timmer and S. Klein [6]. The correlation between ion channel current values has been studied without distinguishing whether they belong to the same state of the channel: open(state of a high current); open inactive (state of a low current); closed; or to other different states with the use of dwell time cross-correlation functions to study single-ion channel gating kinetics. The correlation

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within the ion channel current time series has been checked directly by calculating the auto-correlation function $k(t)$, which measures the memory existing between time delayed values of the ion current [7,8]. Existence of memory in ion channel was studied by Fang ji-quan et al. [9].

A few techniques have been developed for analyzing nonlinear 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, about 0.73, that is, the phenomena exhibited correlation rather than random time series. 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. 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], that is the successive openings and closings assumed to be a process with memory, similarly literatures [14–16] reported rescaled range method applications. To check whether there is any correlation between continued open and closed states, the problem is tantamount to checking whether the system being in one open(closed) state can 'observe' the previously open (closed) state, even though it is separated by the opposite state. To develop a rather simple stochastic model and intuitive interpretation, the statistic properties of the ion single channel current signals have been explored in our databank and an attribute of memory is found in voltage dependence K^+ single channels.

The main purpose of this paper is to analyze the fluctuations of ion channel current using the statistical analysis method: auto-correlation function. We give 'prescriptions' for analyzing any enough long time series by means of which not only nonlinear character of the investigated current signal is brought to light, but also the statistical characteristics related to it can be seen. Although we did not discuss the possible mechanism of ion transport, the result suggested a nonlinear character of the gating process.

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 DRGs 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 350 °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) [17–19].

2.2. Solutions and electrophysiology

Single channel recording was carried out at room temperature using an EPC-9 patch clamp amplifier (Germany), using the cell-attached configuration of the patch-clamp method. Recording single potassium channel current from DRG, cells were isolated and maintained in Hank's balanced salt solution with the following composition (millimolars) Na Cl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, D-glucose 10. Its osmolarity was adjusted to 340 mOsm with sucrose and pH was adjusted to 7.4 with KOH. Cell-attached bath solution contained (in millimolars) Na Cl 5, KCl 150, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, $CdCl_2$ 0.2, D-glucose 10, pH=7.4. The patch–pipette (internal) solution contained (in millimolars) Na Cl 5, KCl 150, $MgCl_2$ 1, HEPES 10, EGTA 11, its osmolarity was adjusted to 340 mOsm with sucrose and pH was adjusted to 7.4 with KOH. The pipettes had resistances of 8–12 ΩM after backfilling with an internal solution.

Single channel current was recorded with EPC-9(Germany). Current signals were filtered with a cutoff frequency of 1 kHz(eight-pole Bessel) and sampled at 5 kHz, for single-channel analyses, we used data from patches in which only one K+ channel was present.

3. Theory

3.1. Auto-correlation function

The auto-correlation function is used in order to investigate the correlation (memory) in the time series of open and closed fluctuations as shown in Refs. [7–9]. The auto-correlation function $k(i,\ t)$ of the signal $\{X_t\}_{t-1}^{T}$ is defined as

$$k(i,t) = \frac{\langle (X_i - \mu_i)^*(X_{i+t} - \mu_{i+t}) \rangle}{\sigma_i{}^*\sigma_{i+t}},$$

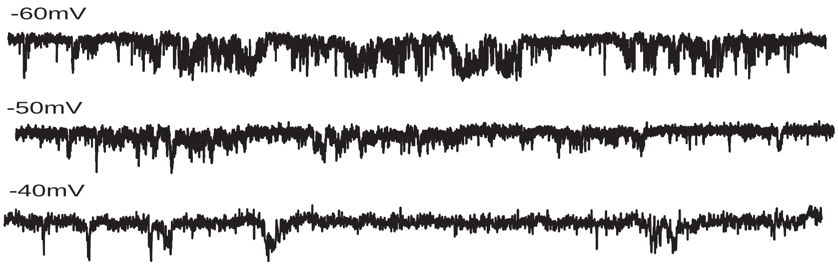


Fig. 1. Shows traces of single-channel currents recorded at different pipette potentials.

where σ_i is the standard deviation and μ_i is the mean value of the sample at the moment i, so σ_{i+t} and μ_{i+t} is the standard deviation and the mean value of the sample at the moment $i+t$. If the investigated time series is stationary, that is, it has the same statistical properties at every moment i, the auto-correlation function is a function of the time lag t

$$k(t) = \frac{\langle X_i * X_{i+t} \rangle - \mu^2}{\sigma^2},$$

σ and μ do not depend on a moment t.

It is used to measure the memory with respect to time span in the series DAT. Obviously, $\boldsymbol{k}(0)=1$ and $-\mathbf{1} \leq \boldsymbol{k}(\boldsymbol{t}) \leq \mathbf{1}$ for any $\boldsymbol{t} \neq \mathbf{0}$.

3.2. Statistical significance of auto-correlation

Denote the population of auto-correlation function with $\boldsymbol{r}(\tau)$ for time span τ. It can be proved that for white noise, $\boldsymbol{r}(\boldsymbol{\tau})=\mathbf{0},\ \boldsymbol{\tau} \neq \mathbf{0}$, such that when the length of the series is n ($n+1$ time points), there will be around 95.5% of the time points having their sample auto-correlation falling into the band between $\boldsymbol{r}=\pm \mathbf{2}n^{-1/2}$. Therefore, if there are far more than 4.5% of the time points having their sample auto-correlation out of the band, the population auto-correlation function is regarded as significantly different from $\boldsymbol{r}(\boldsymbol{\tau})=\mathbf{0},\ \boldsymbol{\tau} \neq \mathbf{0}$, hence the attribute of memory exists.

4. Results

The sealing of a glass micropipette onto the surface of a rat dorsal root ganglion neurons results in the appearance of unitary current events in about 75% of the attempts. Fig. 1 shows traces of single-channel currents recorded at $V=-60$ mV, $V=-50$ mV and $V=-40$ mV; three kinds of different pipette potentials. As can be seen, the channels are voltage dependent: the probability of having an open channel (P_0) increases with depolarization.

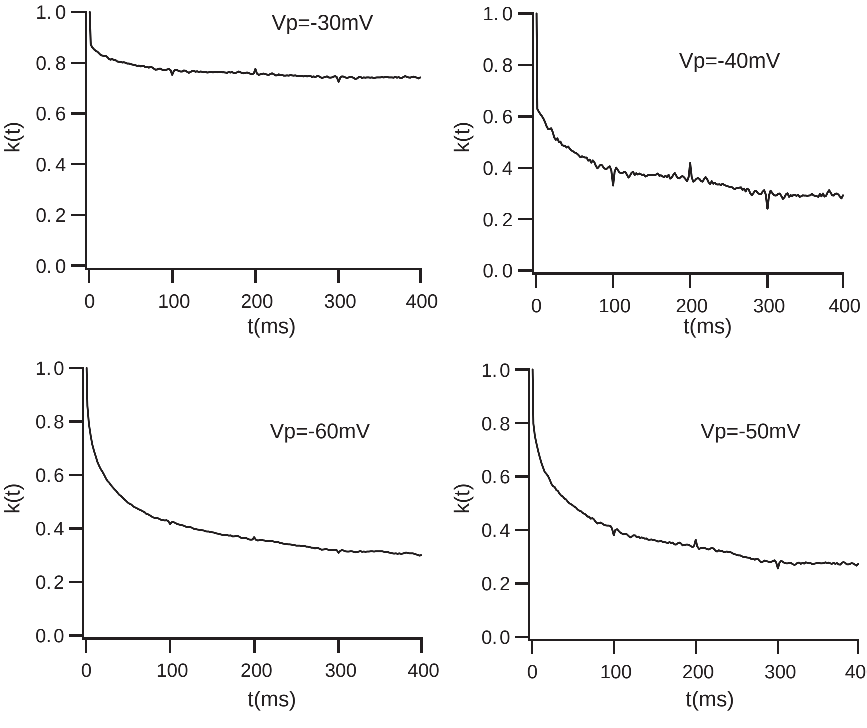


Fig. 2. At different pipette potential auto-correlation functions based on K^+ channel recording.

4.1 Pipette potential difference auto-correlation function

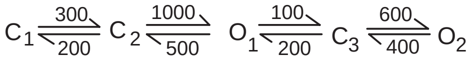
The sample auto-correlation functions $k(t)$ of more than 50 series of patch clamp recordings for rat dorsal root ganglion neurons in our databank has been calculated, the results indicate the existence of memory with $k(t)$ decreasing slowly.

Under the same experimental conditions, shown above are pipette potential differences of auto-correlation functions ($V_p = -30$ mV, -40 mV, -50 mV, -60 mV). One cannot see the similarity among the original signals due to the random feature, but auto-correlation function based on the data shows a well-consistent feature. Fig. 2 showed pipette potential differences of auto-correlation functions based on K^+ channel recording. The results show that pipette potential $V_p = -30$ mV indicated stronger memory; under $V_p = -40$ mV, -50 mV, -60 mV condition, the properties of ion channel memory also exists.

4.2. Auto-correlation functions and fractal dimension

A series double-logarithmic plot of the auto-correlation function $k(t)$ vs time (t/ms) is plotted in Fig. 3. The auto-correlation function can be treated as a measure of memory in the system while its exponents give information on the speed of correlation loss between states separated in time. For $V_p = -30$ mV the auto-correlation function decreases with power laws in all intervals. The power law is on the basis of Eq. (4.1) for all time, the correlation function scales with exponent 0.173 ± 0.04, the observed behavior of the auto-correlation function, revealing the existence memory in the system for all time.

$$k(t) \propto t^{-0.173 \pm 0.04} \tag{4.1}$$



Scheme 1.

For $V_p = -40$ mV the auto-correlation function decreases with power laws in two difference intervals, the power laws are

$$k(t) \propto \begin{cases} t^{-0.15 \pm 0.08} & \text{for} \quad t < 20 \text{ ms} \\ t^{-0.22 \pm 0.04} & \text{for} \quad t > 20 \text{ ms} \end{cases}. \tag{4.2}$$

The first one for $t < 20$ ms, the correlation function scales with exponent 0.15 ± 0.08, a low value of the exponent assures a slow correlation fall. The second for $t > 20$ ms, the correlation function scales with exponent 0.22 ± 0.04, a high value of the exponent shows a fast correlation fall. The similarity for $V_p = -50$ mV and $V_p = -60$ mV the auto-correlation function decreases with power laws in two difference intervals, the power laws respectively are

$$k(t) \propto \begin{cases} t^{-0.150 \pm 0.10} & \text{for} \quad t < 20 \text{ ms} \\ t^{-0.164 \pm 0.02} & \text{for} \quad t > 20 \text{ ms} \end{cases} \quad Vp = -50mV \tag{4.3}$$

$$k(t) \propto \begin{cases} t^{-0.210 \pm 0.06} & \text{for} \quad t < 20 \text{ ms} \\ t^{-0.199 \pm 0.02} & \text{for} \quad t > 20\text{ms} \end{cases} \quad Vp = -60mV. \tag{4.4}$$

For $V_p = -50$ mV, as $t < 20$ ms the correlation function scales with exponent 0.510 ± 0.10, a high value of the

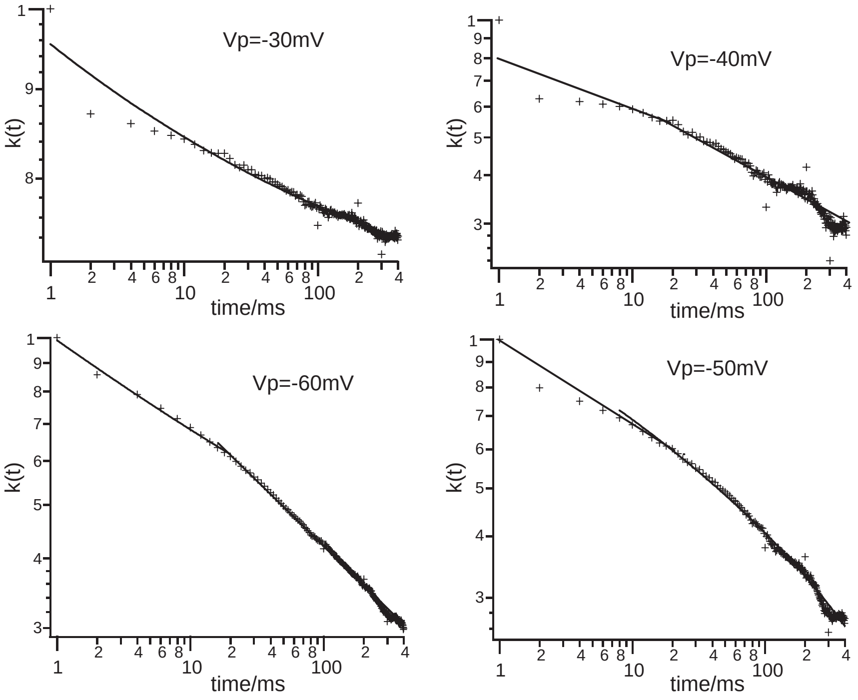
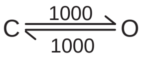


Fig. 3. A series double-logarithmic plot of the auto-correlation function $k(t)$ vs time (t/ms) shown, pipette potential at -30 mV, -40 mV, -50 mV, -60 mV.



Scheme 2.

exponent shows a fast correlation fall, for $t > 20$ ms, the correlation function scales with exponent 0.164 ± 0.02, a low value of the exponent assures a slow correlation fall. For $V_p = -60$ mV, the correlation function scales with exponent of two parts are almost equal.

The literatures, [7] and [22] point out the possibility of using the auto-correlation function in an estimation of the fractal dimension of a time series. The fractal nature of ion channel activity has been described in the literatures [20–22]. For a signal occurring in one dimension, the fractal dimension lies between 1 and 2. the fractal dimension D and the exponent of auto-correlation scaling D_k are related by a simple linear formula[7,22].

$$D = 1 + \frac{D_k}{2}. \tag{4.5}$$

When $1 < D < 1.5$ indicates a positive correlation between the nearest neighbor points, when $D = 1.5$ indicates the system has no correlation, when $D > 1.5$ indicates a negative correlation between the nearest neighbor points. According to above formula, the fractal dimension calculated on the basis of Eq. (4.5), for the difference scaling regions of the auto-correlation function of difference pipette potential is equal to.

$$D = 1.086 \pm 0.02 \text{ for all } t \quad V_p = -30 \text{ mV} \tag{4.6}$$

$$D = \begin{cases} 1.075 \pm 0.04 & t < 20 \text{ ms} \\ 1.11 \pm 0.02 & t > 20 \text{ ms} \end{cases} \quad \text{for} \quad V_p = -40 \text{ mV} \tag{4.7}$$

$$D = \begin{cases} 1.225 \pm 0.05 & t < 20 \text{ ms} \\ 1.082 \pm 0.01 & t > 20 \text{ ms} \end{cases} \quad \text{for} \quad V_p = -50\text{mV} \tag{4.8}$$

$$D = \begin{cases} 1.105 \pm 0.03 & t < 20 \text{ ms} \\ 1.0995 \pm 0.01 & t > 20 \text{ ms} \end{cases} \quad \text{for} \quad V_p = -60 \text{ mV} \tag{4.9}$$

the results shown ion channel current fluctuations that existed positive correlation.

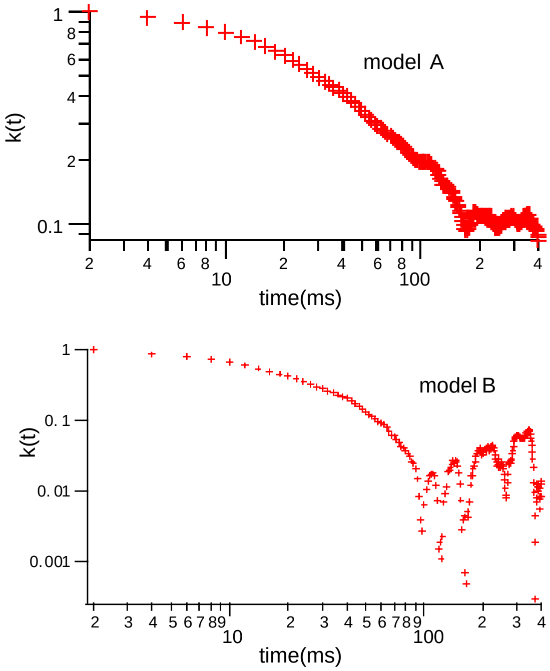


Fig. 5. A series double-logarithmic plot of the auto-correlation function $k(t)$ vs time (t/ms) shown by model A and model B.

4.3. Simulation of single channel currents

We have simulated five-state model A and assumption an underlying Markov chain, states O_1 and O_2 had a conductance level of 1, and three closed states had conductance zero. The mean dwell times in the states O_1 and O_2 are clearly $1.66 * 10^{-3}$ s and $2.5 * 10^{-3}$ s; which we sampled at time interval 0.05 ms, the rate constants and kinetics are shown in Scheme 1.

According to literature [24], we have also used two-state model B to simulate another assumption of an underlying Markov chain, state O had a conductance level of 1, and closed state had conductance zero. The mean dwell times in the states O is clearly $1.0 * 10^{-3}$ s; which we sampled at time interval 0.05 ms, the rate constants and kinetics are shown in Scheme 2.

The sample auto-correlations (Fig. 4) are calculated based on the simulation multiple state Markov models, the

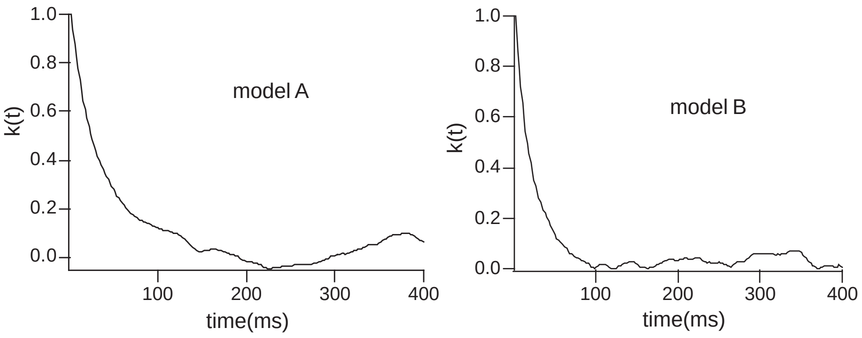


Fig. 4. Auto-correlation functions based on difference model.

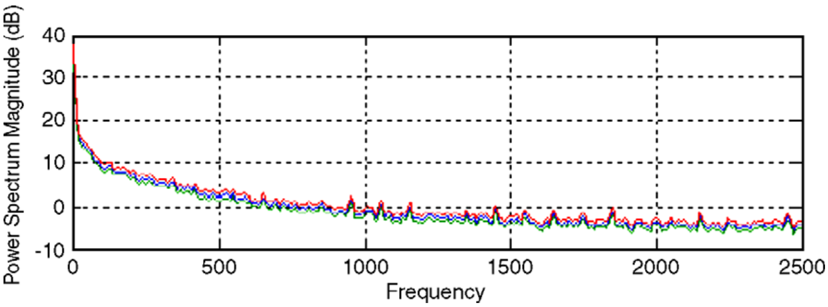


Fig. 6. Shown considerable variability with a broad 1/f -like spectrum.

results show that the correlations in data not only depends on the number of states in the Markov model, but also depends on delay-time. In model A, after a delay-time of 180 ms, the correlation was non-existent; while in model B, after a delay-time of 100 ms the correlation was also non-existent (Fig. 5). At one hand, in specific multiple state Markov models that might have existed weaker memory, the memory decreases with time slowly. On the other hand, the correlation of ion current fluctuation decreases with time and may have something to do with random processes.

4.4. Spectral analysis of ion channel current

As white noise produced a large independence factor, its power spectrum magnitude had nothing in relation with frequency, its power spectrum continued to be a flat spectrum. For chaos, as it was non-periodic, its power spectrum also was continuous, but chaos movement was extremely complex, thus chaos's spectrum was not a flat spectrum. Fig. 6. shown ion channel signal considerable variability with a broad 1/f -like spectrum with $\alpha = 1.261 \pm 0.24$ for pipette potential at -60 mV, which differs significantly from 2, the latter value being typical for the Markov processes.

5. Discussion

Although Markov model was widely used to represent the ion channel kinetics, and interpreted the results from single channel and noise analysis experiments, The channel gating kinetics have been assumed to exist in a finite number of discrete 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. However, it is known that the spontaneous fluctuations in the conformation of proteins involve many different processes that occur over many different time scales from 10^{-15} to 10^3 s [21,23].

Therefore the gating process, at least the one analyzed in this paper, seems to be a result of both random noise and of some deterministic process, possibly chaotic with fractal characteristics. We have examined different pipette potential ion channel current fluctuation auto-correlation character, the different regions of the auto-correlation function with different power law decays have been clearly distinguished. The result indicated the existence of memory in the system. At the same time, it seems that the attribute of memory may also be voltage dependent. The specific multiple state Markov models were studied and the data was simulated. The results give evidence that in the ion current fluctuation, there exists a weaker memory, and the correlation decreases slowly with the time. However, it is propitious to analyze exactly ion channel kinetics, on the other hand, spectral analysis of ion channel current results was also indicative that its power spectrum is continuous and its spectrum was not a flat spectrum. Ion channel current signal shows considerable variability with a broad 1/f -like spectrum, with $\alpha = 1.261 \pm 0.24$. The study results indicated that it was described the gating kinetics of k^+ channel in rat dorsal root ganglion neurons with nonlinear Dynamics properties. Thus above results show that ion channel current fluctuation had nonlinear character. As a specific property that the memory of ion channel has not been widely noticed, it may exist in some ion channel under certain conditions.

This paper is but preliminary with respect to modeling of the ion channel gating kinetics. Its aim is to provide the means for the analysis of fluctuation properties of ion channel currents. Deeper understanding can be gained by comparing results of such analyses for various channels in various conditions. The question, which appears in this context is, what is the physical phenomena and possible mechanism of memory in ion channels? One has to take into account: if it happens to some channels, how to classify and identify systemically, how to build a model? Containing both stochastic and deterministic (mechanistic) components. All these study should be the future direction of research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2005.05.005

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