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Nonlinear determinism of spiking activity recorded from rat *suprachiasmatic nucleus* neurons in vitro

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Abstract

The possible existence of nonlinear determinism in complex interspike interval (ISI) patterns of rat suprachiasmatic nucleus (SCN) neurons is investigated in hypothalamic slice preparations. ISI sequences are recorded from 173 neurons using a cell-attached patch recording technique, and their correlation dimensions (D_2) are estimated. These values are then compared with those of the randomly shuffled surrogate data. Among 173 neurons, 16 neurons are found to exhibit deterministic ISI patterns of spikes. We show, using clustering analysis, that SCN neurons is divided into two subgroups of neurons each having distinct values of skewness (SK) and coefficient of variation (CV): a group of irregularly spiking SCN neurons having large values of SK ($2.0 < \mathrm{SK} < 10.0$) and CV ($0.4 < \mathrm{CV} < 0.8$) and the other group of regular SCN neurons with smaller SK ($-1.0 < \mathrm{SK} < 2.0$) and CV ($0.1 < \mathrm{CV} < 0.4$) values. Interestingly, most deterministic SCN neurons ($\frac{14}{16}$) belong to the group of irregularly spiking neurons.

Keywords: Suprachiasmatic nucleus; Nonlinear determinism; Heterogeneity

1. Introduction

The superchiasmatic nucleus (SCN) contains pacemaker neurons imposing circadian rhythmicity in mammals [7,8]. The circadian rhythm of SCN neurons is expressed by the slow sinusoidal modulation of their mean firing rates. This modulation is closely associated with rhythmic changes in endogenous and behavioral activities of the

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mammalian brain and body. Unlikely the slowly varying mean firing rates, interspike intervals (ISIs) of SCN neurons are highly complex, often accompanied by intermittent bursts of spikes [9]. So far, the relationship between the complex sequence of action potentials operating on a fast time scale and the mean firing rates varying over a much slower time scale is unknown.

To gain some insights into this question, it is quite necessary to characterize the complex spiking sequences of SCN neurons. In this study, we used nonlinear time series methods to determine whether the complex ISI patterns of SCN neurons are truly random or deterministic, a critical issue for understanding its underlying cellular mechanism. Nonlinear dynamical theory has shown that systems of deterministic chaos can exhibit a very irregular behavior as complex as stochastic systems do. Indeed, a number of recent studies have demonstrated that irregular spiking activities recorded from various brain regions emerge from a deterministic chaos [1,6]. To our knowledge, this is the first study investigating the spiking patterns of SCN neurons based on nonlinear dynamical measures.

2. Methods

Male Sprague-Dawley rats ($n=52$; 40–100 g) were housed in a temperature-controlled room (22–24°C) under a 12/12-h light/dark cycle (light on 07:00–19:00) for at least 2 weeks prior to use. The rats were anesthetized with Nembutal (6 mg/100 g body weight) in the daytime of subjects, and then the brains were quickly removed and submerged in ice-cold artificial cerebrospinal fluid. Using a vibrating tissue slicer (Vibratome 1000, Technical Products International, USA), a block of hypothalamic tissue was cut into slices coronally at the thickness of 120–150 μm. The experimental procedures described above were in accordance with the guideline set by the Korea University College of Medicine Animal Research Policies Committee.

After 1-h incubation in the recording chamber, extracellular recordings were commenced at room temperature (25–27°C). The recording electrodes made of borosilicate tubings (Sutter Inst. Co. USA) had a tip diameter of 2–4 μm with a resistance of 3–5 MΩ. Cell-attached patch (CAP) configuration without membrane rupture was achieved for extracellular, single-unit recording. In a CAP mode, a single action potential caused a transient capacitive current over the patch of membrane sucked into the pipette tip. This was recorded under voltage clamp conditions with a pipette potential of 0 mV. The recordings were performed using Axo-patch 200B amplifier (Axon Instruments, USA) in track mode from 173 SCN neurons for 20–40 min, and the ISI data were stored using pClamp software. The mean number of data points was 7356 ± 2961 (range: 1422–19 213).

The D_2 reflects the number of independent variables that are essential for describing the dynamics of the concerned system. In general, the larger the value of D_2, the more complex the behavior of the system. The D_2 values of ISI sequences were estimated using the Grassberger–Procaccia algorithm [2]. The D_2 of a deterministic system is preserved as the embedding dimension increases, while that of a stochastic system increases without any saturation.

The surrogate data test was used to confirm the presence of deterministic nature in the ISI sequences of SCN neurons. Surrogate data are a randomized sequence of the original data with all nonlinear determinism destroyed [10]. A statistically significant difference in the D_2 values between the original data and the surrogate data would indicate the presence of nonlinear determinism in the original data. Pairwise t-tests of the difference between the D_2 values of the original ISI sequences and the mean D_2 values of their 19 surrogates were applied to test the null hypothesis of a stochastic behavior.

3. Results

All observed neurons ($n = 173$) produced a highly irregular ISI sequence, whose distribution was unimodal with a mean of 0.24 ± 0.09 s. D_2 values were estimated for all data sets as the embedding dimension (d_e) increased from 2 to 15. Among 173 neurons, 16 neurons exhibited saturated D_2 values as the d_e increased, indicating that the behavior of this neuron was quite deterministic with finite degrees of freedom, despite the high irregularity of the ISI sequence. The mean D_2 values of the 16 deterministic neurons were 8.15 ± 1.36. The D_2 differences between the original data and their surrogate data increased as the d_e increased (Fig. 1(a)). The other neurons (157/173) exhibited truly random ISI patterns. Fig. 1(b) presents that D_2 values for the original ISI data and their surrogate data showed no significant differences as the d_e increased.

Based on the histogram and regularity analysis of ISIs, we test a possibility that there are two subgroups within the populations of SCN neurons. The regularity in spontaneous firing patterns was quantified by the coefficient of variation (CV) in spike intervals, which is defined as the standard deviation divided by the mean ISI. Higher CV values of ISI sequences indicate more irregular firing patterns. Fig. 2 clearly demonstrates that SCN neurons are divided into two clusters in the parametric space of CV and SK: the group with skewed ISI histograms towards more long ISIs ($2.0 < \mathrm{SK} < 10.0$) and high CV values ($0.4 < \mathrm{CV} < 0.8$), which was denoted as 'cluster I', and the neurons with normally distributed ISI histograms ($-1.0 < \mathrm{SK} < 2.0$) and low CV values ($0.1 < \mathrm{CV} < 0.4$), which was labeled 'cluster II'. We quantitatively examined the presence of two subgroups using a hierarchical clustering analysis. The significant differences in CV and SK values between two clusters was confirmed, in Table 1 ($P < 0.001$; Student t-test). Surprisingly, the majority of deterministic SCN neurons (14/16) belonged to cluster I. These findings indicate that SCN neurons are heterogeneous in properties of firing patterns.

4. Discussion

A deterministic (or predictable) temporal structure of ISI sequences has been repeatedly reported in various neuronal preparations [3,4]. In fact, our present study demonstrated that some SCN neurons also generate deterministic ISI sequences. In other words, interspike intervals and overall patterns of neuronal spike trains are

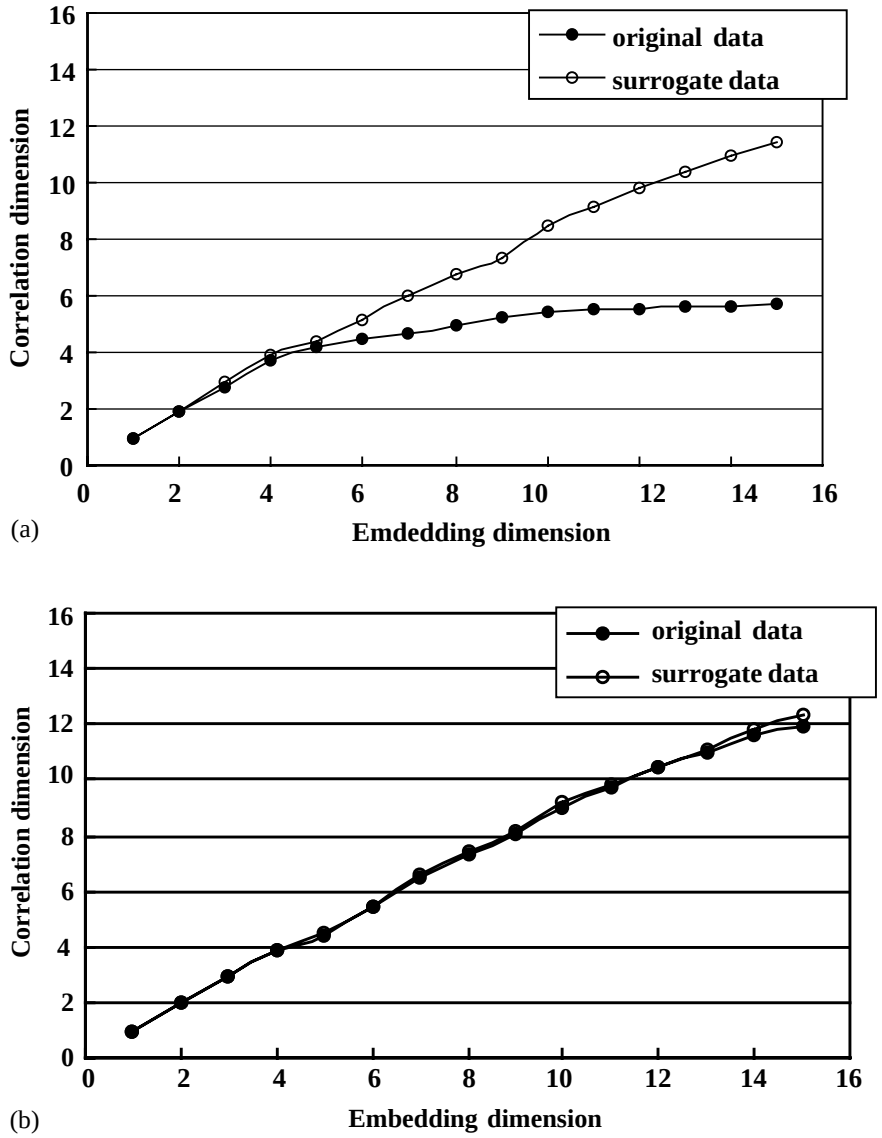

Fig. 1. The average D_2 values of the original ISI data recorded from (a) a typical deterministic SCN neuron and (b) a stochastic SCN neuron and their surrogate data as a function of the embedding dimension.

often generated and/or exquisitely regulated by deterministic rules, regardless of their highly irregular and complex behavior. This can be quite significant for SCN neurons that play a critical role for imposing circadian rhythms of physiological functions and behavior by providing modulated output signals to target brain regions through neural efferents or in a diffusible manner [5]. For the periodic modulation in the spiking

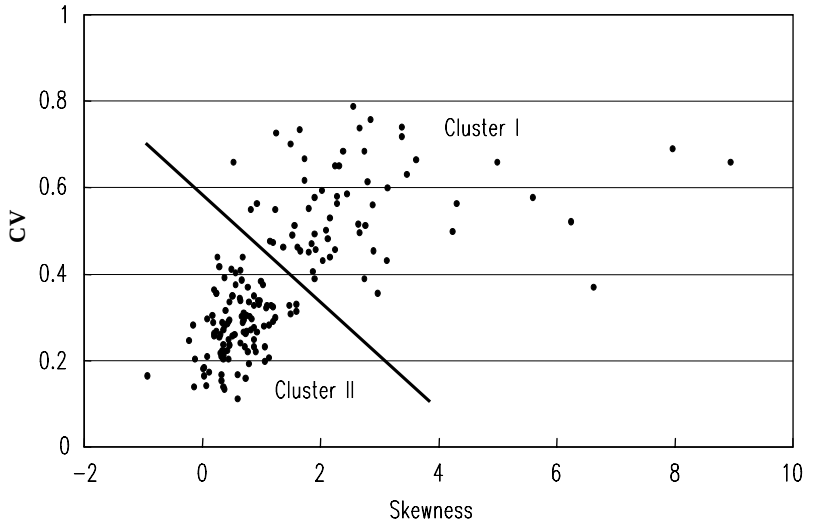

Fig. 2. Plot of CV of interspike intervals versus SK for 173 SCN neurons. Two clusters can be discerned having small CV and SK values, and large CV and SK values, respectively. The values on the axes are not standardized.

Table 1
Spike train properties (mean ± standard deviations) per cluster of SCN neurons ($n = 173$)

Parameters	Cluster I	Cluster II	t-Value
Number of cells	60	113	—
Mean ISI (s)	0.33 ± 0.14	0.21 ± 0.06	6.14*
Skewness	2.64 ± 1.76	0.67 ± 0.86	7.18*
CV	0.55 ± 0.12	0.26 ± 0.08	13.92*
Mean D_2	12.11 ± 3.54	14.56 ± 1.56	−4.59*
Probability of deterministic neurons	14/60	2/113	—

Student t-test; CV: coefficient of variation. *: $P < 0.001$.

rates, individual SCN neurons should be able to monitor spike trains at every moment and control overall patterns of spikes on a short time scale. Thus, we may speculate that SCN neurons must employ a deterministic rule to regulate interspike intervals to impose circadian rhythmicity on their overall firing patterns.

The other interesting finding is that SCN neurons are heterogeneous. This result is quite consistent with the earlier findings of Pennartz et al. [9]. They have examined electrophysiological and morphological properties of SCN neurons in hypothalamic slices to find that SCN neurons can be partitioned into three clusters. Although we cannot compare all the electrophysiological properties of the clusters in our data with those of Pennartz group, the corresponding clusters have similar ranges of mean firing rates and CV values. The heterogeneity of nonlinear determinism in SCN neurons suggests that the SCN is composed of distinct neuronal subgroups each of which makes

a unique functional contribution to the circadian timing. For further investigation, the biochemical, electrophysiological, and morphological properties of deterministic SCN neurons will be examined to improve our understanding of the functional roles of distinct groups of SCN neurons.

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