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NONLINEAR DETERMINISM OF THE SPIKING ACTIVITY RECORDED FROM *SUPRACHIASMATIC NUCLEUS* NEURONS OF RATS IN VITRO

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Possible existence of nonlinear determinism in the spiking activity of the rat Suprachiasmatic nucleus (SCN) neurons in vitro is investigated. The correlation dimensions of ISI data recorded from 173 SCN neurons are estimated and compared with those of the surrogate data. 16 neurons (16/173) show a clear evidence of nonlinear determinism. To examine if an origin of the nonlinear determinism in the SCN neurons is the interaction between neurons, we apply bicuculline to 56 SCN neurons to block inhibitory synaptic connections. A deterministic structure appears after the bicuculline application in 8 previously stochastic SCN neurons, suggesting that nonlinear determinism in SCN neurons originate not from neuronal interactions, but from an intrinsic property of single cell dynamics.

(Oral presentation preferred)

SUMMARY

The Suprachiasmatic nucleus (SCN) contains pacemaker neurons imposing circadian rhythmicity in mammals. The circadian rhythm of SCN neurons is mainly expressed by a slow modulation of their spontaneous firing rates even in vitro and in culture as well as in vivo. This circadian modulation is known to be associated with rhythmic changes in endogenous and behavioral activity in mammals [1]. From a physical point of view, the firing activity of SCN neurons on a long time scale is a deterministic oscillation with a period of about 24 hours.

However, interspike interval (ISI) firing patterns of SCN neurons on a short time scale are highly irregular and complex. How SCN neurons generate complex spike patterns and modulate the firing rate periodically by regulating the irregular interspike intervals are unknown. Whether the complex ISI firing patterns of SCN neurons are truly stochastic or not is intricate, because nonlinear dynamical theory has shown that the irregularity of a time series may not necessarily result from stochastic processes [2]. Systems of deterministic chaos show highly irregular and complex behavior like stochastic systems due to such systems' sensitive dependence on initial conditions. Thus, the aim of this study is to investigate if ISI firing patterns of SCN neurons in vitro on a short time scale are deterministic or truly stochastic using nonlinear dynamical methods.

EXTRACELLULAR RECORDING IN VITRO

Male Sprague-Dawley rats (40-100g) were housed in a temperature-controlled vivarium under LD cycle (light on at 7a.m. and off at 7p.m.) for at least two weeks prior to use. During the daytime of subjects, rats were anesthetized with Nembutal (1ml/600g), decapitated and the brains were quickly placed in oxygenated ice-cold artificial cerebrospinal fluid (ACSF). A block of hypothalamic tissue was cut and sectioned with a vibrating tissue slicer (Vibratome) to thickness of 120-150μm in ice-cold ACSF. Coronal tissue slices containing the SCN were transferred to a recording chamber.

Extra-cellular recordings of spontaneous spiking from 174 neurons of 26 rats were performed with Axo-patch clamp 200B. After about 1 hour incubation in recording chamber, all recordings were performed at room temperature (25-27℃). First two minute data was discarded and 20-60 minute recording (mean number of data points: 6,287±1,565) was stored using pClamp software (8.0.2.113 version). For the bicuculline application experiment, 30μM-bicuculline was added to ACSF and 20-40 minute recording was performed after the bicuculline treatment.

NONLINEAR DYNAMICAL ANALYSIS

Nonlinear determinism requires that the trajectories of the state of a system are predictable and can be described by nonlinear differential equations. The correlation dimension (D2) reflects the number of independent variables that are necessary to describe the dynamics of the system. A deterministic system has a finite D2 value, whereas the D2 of a stochastic system is theoretically infinite. The attractor, which is a bundle of dynamical trajectory in the phase space, is reconstructed from the observed ISI sequences by plotting delay coordinates [2]. In our analysis, the D2 of the attractors was evaluated using the Grassberger-Procaccia Algorithm [3]. We estimated the D2 of the data as the embedding dimension d_{emb} (the dimension of the phase space) increases. A deterministic system has finite D2 values as the d_{emb} increases, whereas the D2 value of a stochastic system increases without any saturation along with the d_{emb}.

Surrogate data method was also used to help detect nonlinear determinism. Surrogate data are linear stochastic time series that have the same power spectra as the raw time series.

They are randomized to destroy any deterministic nonlinear structure that may be present. Statistical difference of the D2 between the raw data and its surrogate data indicates the presence of nonlinear determinism in the raw data.

RESULTS AND DISCUSSION:

Nonlinear dynamical analysis demonstrated that sixteen SCN neurons (16/173) had saturated low D2 values (mean and S.D.: 8.92±1.35) as the d_{emb} increased, whereas the D2 values of their surrogate data increased without any saturation. This indicates the presence of nonlinear determinism in the data. Remaining 158 neurons, however, had increased D2 values as the d_{emb} increased, implying no presence of determinism.

In addition, we found that SCN neurons were heterogeneous in electrophysiological properties including ISI distributions, the regularity and determinism. Interestingly, a group of irregular SCN neurons having larger skewness and coefficient of variation (CV) values had a tendency to be deterministic (14/60 neurons). The other group of regular SCN neurons (smaller skewness and CV values) was more stochastic (only 2 out of 113 neurons) as shown in Table 1. These results are, in part, consistent with findings by Pennartz et al. [4] that SCN neurons are heterogeneous in active membrane properties and can be partitioned into the regular and irregular firing clusters.

In order to investigate if an origin of nonlinear determinism in ISI data of SCN neurons is the interaction between neurons through synaptic coupling, we applied bicuculline, the $GABA_A$ receptor antagonist, to 56 SCN neurons to block inhibitory synaptic connections between neurons. Eight previously-stochastic SCN neurons appeared to have a deterministic structure after the bicuculline treatment. Additionally, a deterministic structure of the ISI data from one deterministic SCN neuron was not destroyed by the bicuculline treatment.

Furthermore, D2 values of the ISI data significantly decreased after the bicuculline application (Table 2). These results suggest that nonlinear determinism in SCN neurons may originate not from neuronal interactions through GABAergic synaptic transmission, but from an intrinsic property of single cell dynamics, or neuronal interactions through non-GABAergic synaptic transmission, or non-synaptic communication.

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TABLE 1. Spike train properties per cluster of SCN neurons (n=173).

Parameters	Cluster 1	Cluster 2	t-value
Number of cells	60	113	-
Mean ISI (sec)	0.33±0.14	0.21±0.06	6.14*
Skewness of the spike distribution	2.64±1.76	0.67±0.86	7.18*
CV of spike intervals	0.55±0.12	0.26±0.08	13.92*
Average D2	12.11±3.54	14.56±1.56	-4.59*
Probability of deterministic neurons	14/60	2/113	-

Student t-test

*: $P<0.001$

TABLE 2. Changes in spike train properties of SCN neurons by bicuculline application (n=56)

Parameters	Control state	Bicuculline state	t-test
Mean ISI (sec)	0.18±0.06	0.20±0.19	-0.85NS
Skewness of the spike distribution	3.43±9.02	9.84±14.83	-3.03**
CV of spike intervals	0.24±0.17	0.95±1.47	-2.77**
Correlation dimension (D2)	14.15±1.19	12.93±2.69	2.28*

Paired t-test

NS: not significant *: $P<0.05$ **: $P<0.01$