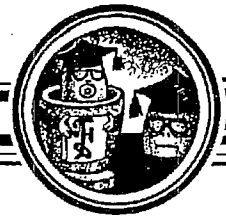

Изв. вузов «ПНД», т.12, № 1-2, 2004

NEURODYNAMICAL CONTROL OF HEARTBEAT IN SPINY LOBSTER, *PANULIRUS JAPONICUS*

Toru Yazawa, Ken Kiyono, Katsunori Tanaka, Tomoo Katsuyama

We performed time series analysis of heartbeat interval of Japanese spiny lobster. We studied isolated and intact hearts. The fluctuation pattern of beat-interval was different in the two conditions. However, the power spectra derived from two conditions were not distinguishable. We, therefore, considered that those spectra might be contaminated by unsteady «trends» in constantly changing internal and external environments. In order to remove such potential trends, we applied detrended fluctuation analysis and obtained distinguishable scaling exponents between isolated and intact hearts: 0.51±0.02 and 0.8±0.2, respectively. Those exponents indicate that fluctuation of isolated hearts have white noise-like qualification, while that of intact hearts have a *1/f* - like or a long-range correlation nature. These results suggest that, by using the scaling exponent, it may be possible to determine whether or not the heart has lost contact with the central nervous system, provided the exponent is derived from a large sample, for example about 10,000 beats.

Introduction

Heart rate variations reflect the complex control of the heart from the cardiac control center in the brain [1]. Beat-to-beat interval variations, therefore, contain information about the activity of cardio-regulatory neurons. The heart of lobster receives innervations of only three kinds of cardio-regulatory axons from the brain center [2]. This simplicity may have a great advantage for studying physiological significance of heart rate variations. To understand neurodynamical control of heartbeat, we performed time series analysis of heartbeat intervals of Japanese spiny lobsters.

When one considers the brain-heart system as a complex dynamical system, *scaling* property (such as power spectrum, scaling exponent, etc) one needs a way to characterize the system quantitatively. Spectral analysis has been widely used for approaching such a goal [3]. However, we have previously noticed that the pattern of fluctuation differs between the hearts in different conditions, although the power spectra were almost identical [4]. Since biological systems usually have trend, we must consider that Fourier-spectral analysis is invalid for such biological data that have trend. To remove the trend, we adopted the detrended fluctuation analysis (DFA) [5]. This test revealed a difference in the scaling exponents between isolated and intact hearts. We here demonstrate the possibility to distinguish between states of heartbeat by means of the scaling exponent.

Materials and Methods

Physiology. Spiny lobsters, *Panulirus japonicus*, were obtained from a fisherman at Shimoda, Japan, and maintained in an aquarium. The isolated hearts were maintained in a steady physiological condition (20 C, lobster saline solution [4]) and heartbeats were recorded with a mechanical force transducer (see ref [6] for the method). In the intact hearts, electrical activity of cardiac muscle (ECG) was recorded by a conventional electrophysiological method (see ref [4]). In both cases, heartbeat signals were sampled at 1 KHz and stored in the PC and analyzed using the Power Lab (ADI, Australia) and our own program. The period of length of recordings was usually 2 to 3 hours, about 10,000 beats. Seawater was effective for isolating the ECG from environmental electrical noise. The implanted electrodes did not seem to adversely affect the animals. Some implanted lobsters lived more than one year and two animals even ecdysed.

Numerical Analysis. Time interval T_n between nth beat and n+1st beat was calculated from the ECG data. Time-series obtained, $\{T_n\}$, n=1,2,...,N, were analyzed. Peng et al. [5] described DFA test before. We referred those articles for the methods. Briefly, DFA was carried out as follows: at first, we made a new series defined as

$$\{T'_n\} = \{T_n\} - \langle T_n \rangle, \tag{1}$$

where T_n is time interval of the original time series of heartbeat recording, and $\langle T_n \rangle$ is its mean value. Then, summing up $\{T'_n\}$ from the first term to the kth term, we obtain $y(k)$ as follow,

$$y(k) = T'_1 + T'_2 + \ldots + T'_k. \tag{2}$$

Subsequently, we divided $y(k)$ into sub series, which has l terms, and approximated each subseries to the linear function $y_l(k)$ using the least mean square method. Each approximate-function $y_l(k)$ represents a local «trend» of entire series. If the cause of unsteadiness of original data comes only from such a trend, subtraction $y(k)-y_l(k)=\Delta_l(k)$ yields a steady series. Here, it should be noted that this procedure would not remove every types of unsteadiness. The mean square deviation of $\Delta_l(k)$, $F(l)$, estimates the performance of the approximation. If the signal T'_n is self similar, $F(l)$ satisfies the following scaling relation:

$$F(l) \sim l^{\alpha}. \tag{3}$$

Since we are interested in the heartbeat fluctuation against *time* instead of beat number n, we transform the time series $\{T_n\}$ into the function of time, $T(t)$, using the re-sampling method [4]. In this case, the scaling relation (3) is replaced to

$$F(\tau) \sim \tau^{\alpha}, \tag{4}$$

where we introduced time-length τ instead for sub series length l. The scaling exponent α obtained from (4) is related to the exponent of power spectrum. If $F(\tau)$ satisfies (4), power spectrum of the function $T(t)$ forms

$$P(\nu) \sim 1/\nu^{\beta}, \tag{5}$$

where ν is frequency. The power β is related with the exponent α as

$$\beta = 2\alpha - 1. \tag{6}$$

When $T(t)$ is a white noise, since β=0, relation (6) gives α=0.5. For a $1/f$ fluctuation, β=1 and α=1. When α falls in the range $0.5<\alpha<1$, one can say $T(t)$ has a «long-range correlation». We plotted ln ($F(\tau)$) against ln(τ) (see below), and the slope shows the exponents α.

Results

Power spectral density. Heart rate of lobsters fluctuates, but isolated hearts and intact hearts exhibited different fluctuation patterns when comparison was carried out at the same temperature, at the same time of day, the same season of the year, and with a similar body-size of preparation (150±12g). Fig. 1 shows examples of data obtained from ECG for about one and half hour. In heart rate plots, fluctuation of baseline rate is discernable (*a*1 and *b*1). At the baseline level, isolated hearts (*a*1) exhibited greater fluctuations than intact hearts (*b*1). In turn, isolated hearts exhibited much less rate variability than in intact hearts because several spine-like structure can be recognized in intact trace, i.e., data points greater than noise data points. This means that several beats out of 1600 deviated from mean value. One can also notice that the mean rate of intact heart is higher than that of isolated heart (mean interval of 0.5 ms and 1.0 ms, respectively).

Although time series data showed such differences between the hearts in different conditions, power spectral density (PSD) did not exhibit much difference between each other (*a*2 and *b*2), due to potential contamination by unsteady «trend». We thus tried to

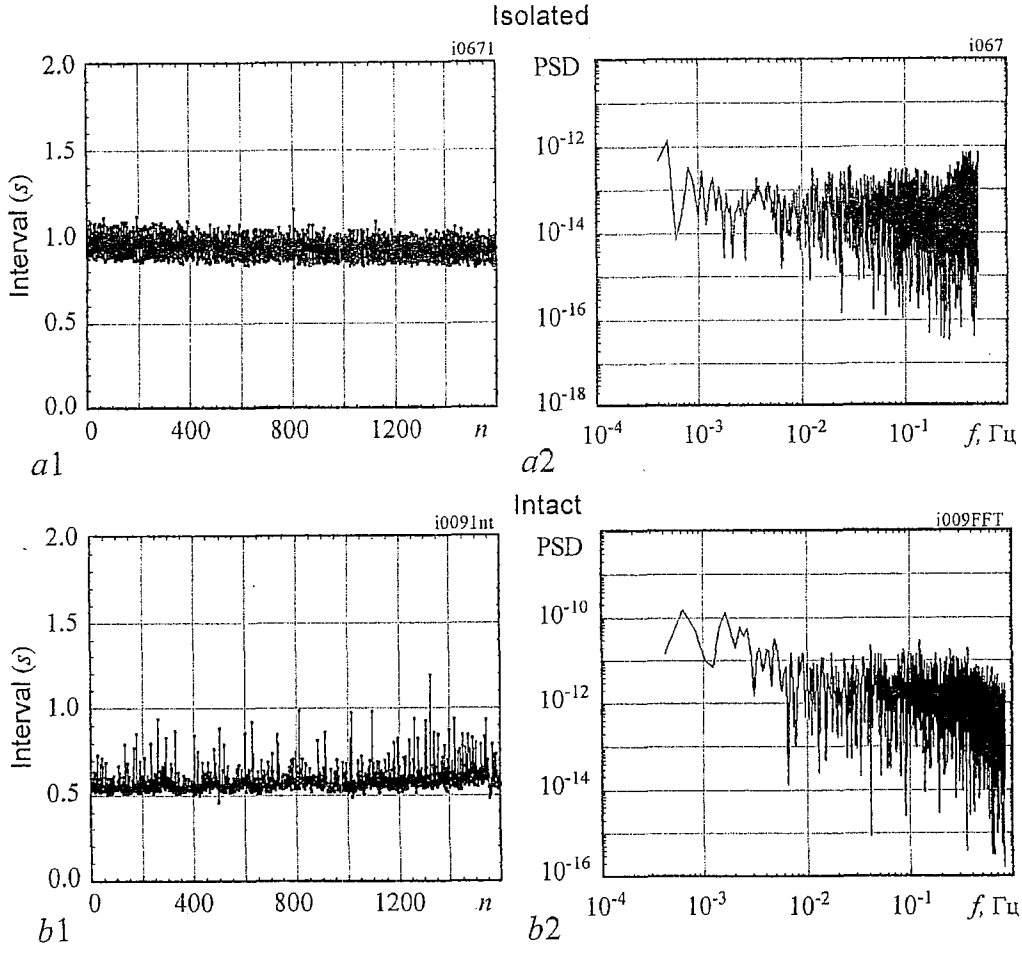

Fig. 1. An example of ECG analysis for isolated (*a*) and intact (*b*) hearts. (*a*1 and *b*1) Representation of time series of heartbeat interval. X-axis (*n*), consecutive heartbeats numbered from 0 to 1600. Y-axis, heartbeat interval shown in sec. Mean intervals are roughly 1 s for isolated heart (*a*1) and 0.5 s for intact heart (*b*1). (*a*2 and *b*2) Representation of power spectral density (PSD) calculated from time series data shown in left hand side respectively. *a*2 and *b*2 are indistinguishable though *a*1 and *b*1 are apparently different in shape

remove the trend. In the present study, we adopted the DFA to estimate scaling exponents [5].

Isolated heart DFA. We next attempted to distinguish heart conditions quantitatively with the DFA. The DFA results also enabled us to give interpretations for the cardiac control mechanisms in terms of physiology.

Isolated hearts showed an exponent of 0.51±0.02 (Fig. 2, *a*). Curiously, the slope was drastically changed at the border of τ=200 s (see abbesses 2.3) between 0.5 and 1.6 in slope. This seems to show an existence of two scaling exponents, α_1=0.5 (τ<200s) and α_2=1.6(τ>200s). In the analysis, we approximated the trend to the linear function $y_l(k)$. We performed additional analyses using higher order approximation functions for the linear function $y_l(k)$. Since these analyses also showed the slope 0.5, we conclude the exponent α_1 (0.5) is robust. However, the time-length τ shifted toward much larger value than «200 s». Furthermore, the slope 1.6 was not always observable as in the case of linear function fitting, which is shown in Fig. 2, *a*. It seems inadequate to go through physiological discussion as to the number 1.6 although the number itself has specific meaning because 1.6 indicates Brownian type of fluctuation.

We emphasize the fact that DFA revealed the unique exponent 0.5 and characteristic time-length 200s, period of which were sometimes much longer at high order approximations.

Intact heart DFA. Intact hearts showed a peculiar scaling feature as shown by straight lines in log-log plots (Fig. 2, *b*). We obtained the exponent of 0.8±0.2 (n=7). This indicates that intact hearts exhibit fluctuations that have long-range correlation. Statistically speaking, one heartbeat interval correlates with forth-coming and later-coming heartbeat intervals. Heartbeats happening at a different moment can be statistically connected with each other. The correlation does not constitute a deterministic connection. A certain heartbeat affects other incident happened afterwards.

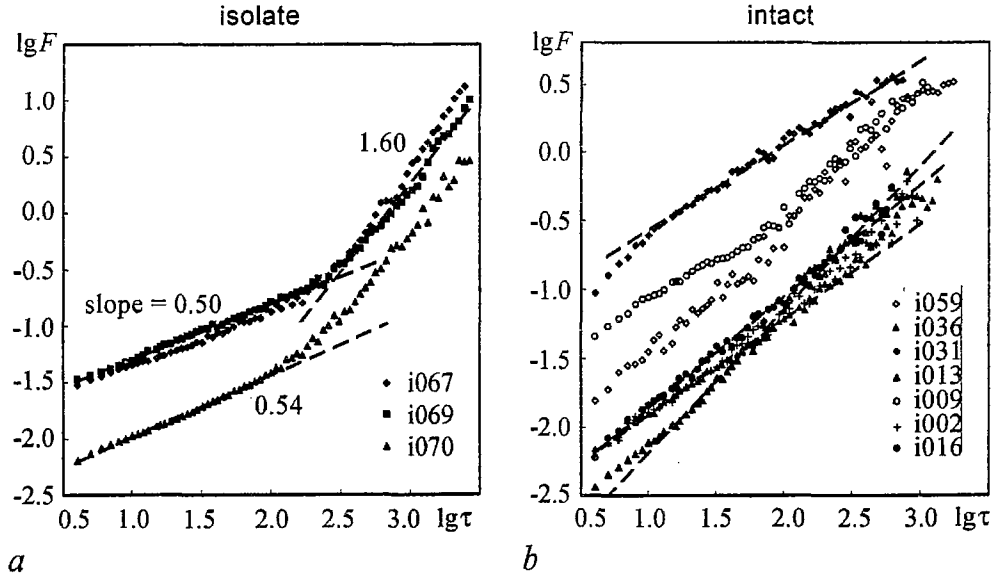

Fig. 2. Log-log plots of F(τ) vs. τ for isolated (*a*) and intact (*b*) hearts. (*a*) All three hearts showed an identical scaling property. In the range τ<200 s, i.e. log τ<2.3, the plots show the slope 0.5. This slope indicates the exponent α of 0.5, which corresponds to a white-noise. (*b*) All seven plots exhibited the exponent about 0.8 in the entire range of τ examined. The α value of 0.8 indicates that the fluctuation has a long-range correlation like the 1/*f* fluctuation

Discussion

Isolated heart. The characteristic period length 200 s appears to be the intrinsic burst rate property of the cardiac pacemaker under the conditions employed here. More remarkably, DFA analysis indicated that the lobster heart can maintain a steady rate for a considerable length of time, 200 s.

What does 200 s mean? We have found from microdialysis (MD)-HPLC analysis of lobster hemolymph that dopamine can be released for the period length of this order, 200 s. When MD-probe-implanted lobsters were disturbed (noxious stimulation applied by rod touching, for instance), the circulating dopamine level increased sharply from resting conditions (5 mg per 10 micro-litter microdialysis sample) after the lobster was disturbed by being touched or by human approach (about 100 pg dopamine per 10 micro-litter). After a sharp peak of 100 pg was attained, the dopamine level decayed exponentially along with a curve of time-constant about 200 s (Yazawa, in preparation in detail). This reveals not only pulse-like hormone release induced by sudden stress but also a short life of effective dose of hormones such as dopamine, which disappear in the blood stream in minutes.

Hormones are believed to bind their receptors on the target cell surface. The binding triggers an intracellular signal transduction cascade, a chemical chain reaction. To accomplish this, target cells must «freeze» until completion of information transmission before executing the state change of intracellular environment. The interval of 200 s may be the shortest biological decoding period for the cardiac ganglion before converting information and establish readiness to new state. It takes time for the pacemaker to respond to central nervous input.

Considering the steadiness of heartbeat, it is necessary to try to explain the persistence of exponent 0.5 for 200s. Steadiness of heartbeat may be physiologically important. How is this steadiness accomplished? Previous studies may provide some answers: the regularity is a property of the cardiac ganglion neuronal network that is made up of only 9 neurons in the lobster [7]. The white noise-like fluctuations must arise from this ganglion. The ganglion is indeed designed to be insensitive to perturbations from outside the ganglion, even to distortion, i.e., potentially violent heart muscle movement, but not to bombardment of hormones and neurotransmitters. The resistance to perturbations and the regularity seems to arise from the tight coupling between the neurons of the network. It is known that the pacemakers are not single cells in lobster heart and in vertebrate heart. We have obtained a DFA scaling exponent of 0.5 from isolated bullfrog heart experiments (Yazawa, Tanaka, Katsuyama, unpublished). In both hearts, pacemaker cells always discharge action potentials in synchrony. In hearts in poor physiological condition, the individual can produce independent rhythms. This will result in cardiac dysfunction, a large human social problem nowadays. If the heart is completely isolated from the body, one can check the heart using scaling exponent to confirm whether it works properly by itself.

Intact heart. We obtained the exponent of 0.8 ± 0.2 (n=7). This indicates that intact hearts exhibit fluctuations that have long-range correlation. This exponent has remarkable difference from that of isolated hearts that exhibited a white noise type exponent, i.e., 0.51 ± 0.02. Physiologically, we consider that this result predicts that one heartbeat interval correlates with forth-coming and later-coming heartbeat intervals. Heartbeats happening at a different moment can be statistically connected with each other.

What kind of mechanisms can offer this correlation? The fundamental conformation of protein molecules, i.e., hardness and flexibility, offers potential sites for the mechanism causing and/or bringing such effects. For instance, in cells there are ionic channels for generating myocardial action potentials and there are various enzymes

involved in signal transductor such as c-AMP. Slight changes within a physiological range of chemical composition and ionic composition of haemolymph may alter the conformation of some of these proteins. It is plausible that the correlations arise from such protein-based relatively slow adaptive changes.

The present results, and our bullfrog data as well, indicate that the white noise-like fluctuations seen in isolated hearts are distorted whenever the organ is innervated and under the control of the central nervous system, i.e., in other word, the exponent 0.5 might be altered from 0.8 when the heart are disconnected from the brain. This hypothesis can be tested with developing hearts, such as the heart in chick embryo [3]. There is also an intriguing data worked out on the crayfish heart by Doyle and McMahon (unpublished) reporting that early embryonic heart is myogenic and insensitive to TTX, but becomes innervated soon after (i.e., early embryonic crayfish hearts have no connection with the nervous system). DFA analysis of such embryonic preparations might be informative.

We speculate that the regularity of heart function might be compromised if it was to be sensitive both to external environmental noise and to internal physiological inputs. If the heart itself could see and respond to the environment directly, brain control would be much. But, the heart located inside the body only see the environment through specific information channels that comes to the heart. The entrances for information are evolutionally established architecture, i.e., chemical hormonal pathway and neurotransmitter-mediated nervous system pathway. The α value 0.5 in isolated heart is shifted to 0.8 by the connection to the CNS through hormones and cardio-regulatory neurons. However, we cannot interpret how this shift is derived in terms of physiology. We can only suppose that the mechanisms for producing α value 0.8 are imprinted on the DNA-structure. Even though we cannot identify the factors that give rise to the scaling exponent, α=0.8, it is interesting that 0.8 is similar to a $1/f$ type fluctuation. Interestingly, in vertebrate heart, a $1/f$ type fluctuation was detected in healthy individuals, although the physiological meaning for that fluctuation is still unknown [8]. Here we demonstrated that DFA analysis can be used to distinguish differences between isolated and innervated hearts. We speculate that the human heart transplantation should be accomplished within the period when the isolated heart can exhibit white noise-like fluctuations. During this period, the heart pacemaker appears to be in a good physiological condition. One is led to wonder whether there would be a change in DFA scaling exponent in transplanted heart were to be re-innervated? Unfortunately, the α value of isolated human hearts is not known.

In conclusion, physicomathematical analytical method applied to the ECG reveals the possibility for describing heart condition, i.e., distinguishing out-of-control from proper-control of CNS. This method is testable for many «hearted» animals including humans, if we have enough basic cardiac-physiological knowledge for the species under studying. Crustacean hearts provide a good model system for investigating the basic mechanism of cardio-vascular control.

Acknowledgement

We thank Jerrel Wilkens for reading manuscript and valuable comments. This study was supported in part by grant from the Japan Ministry of Education, Science, Sports and Culture, № 12048217 (microbrain), № 14540633(H15), and by grant from TMU RigakukennkyukaKachoHaibun № 0300,000 (H14) to TY.

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Received 23.04.2004

The paper includes main results published at first in the Proceedings of the International Symposium «Topical Problems of Nonlinear Wave Physics», Nizhny Novgorod, Russia, 6-12 September, 2003, 1-1, p. 19.

УДК [612.172.2:519.6](075.8)

НЕЙРОДИНАМИЧЕСКИЙ КОНТРОЛЬ СЕРДЕЧНОГО РИТМА КОЛЮЧЕГО ЛОБСТЕРА, «PANULIRUS JAPONICUS»

Toru Yazawa, Ken Kiyono, Katsunori Tanaka, Tomoo Katsuyama

Проведен анализ с помощью временных рядов кардиоинтервалов японского колючего лобстера. Исследованы изолированные и интактные сердца. Характер флуктуационной картины интервалов между сердечными сокращениями различен в этих двух случаях. Однако соответствующие спектры мощности не различались. Предполагается, что эти спектры могут быть «загрязнены» неустойчивыми трендами в постоянно меняющейся внутренней и внешней среде. В целях исключения таких потенциальных трендов был применен detrended fluctuation analysis и получены скейлинговые экспоненты, хорошо различимые для изолированных и интактных сердец: 0.51±0.02 и 0.8±0.2, соответственно. Эти экспоненты указывают на то, что флуктуации изолированных сердец изменяются по закону белого шума, а неповрежденных - по закону 1/*f* или имеют характер длительных корреляций. Эти результаты позволяют сделать вывод о возможности

использования скейлинговых экспонент для определения возможных нарушений связи сердца с центральной нервной системой при условии, что экспоненты определены по длительной выборке, например, по 10,000 сердечных сокращений.

Toru Yazawa - Ph.D. Assistant Prof. of Biological Sci. Leader of BiPaC. A physiologist, recently started recording and analyzing heartbeat of humans, frogs, crustaceans, insects, and vervet worms too, because fundamental cardiac control system must have evolved on the Earth from such lower animals.

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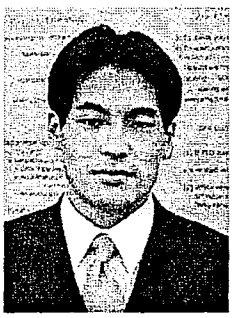

Katsunori Tanaka - Young Physicist, MS. A BiPaC member with powerful skill for physical analysis of heartbeat. His presentation entitled State-Space Representation of Frog Heartbeat was awarded from the Japan Society of Bio-Imaging 2002.

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Tomoo Katsuyama - Ph.D. Professor of Numazu National College of Technology. A statistic Physicist. The interdisciplinary research group, BiPaC, has been established by Toru Yazawa and Katsunori Tanaka in 2002 with a pivotal contributions of Katsunori Tanaka especially serious approach from Physics side to biological phenomena.

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