博士論文 (要約)

論文題目 Mathematical modeling for synchronization of cardiac muscle cells

(心筋細胞の拍動同期現象に関する数理モデル)

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Mathematical modeling for synchronization of cardiac muscle cells

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Abstract

Community effect of cardiomyocytes is investigated in silico by changing number and features of cells as well as configurations of networks. The theoretical model is based on experimental data and accurately reproduces the recent experimental results about coupled two cultured cardiomyocites that proved the fact that the synchronized beating of two coupled cells is tuned not to the cell with faster beating rate but to the one with more stable rhythm. In a network of cardiomyocytes, not a high frequency cell but a cell with low fluctuation becomes a pacemaker and stabilizes the beating rhythm. Beating fluctuation rapidly decreases with increase of the number of cells, N, almost irrespective of the configuration of the network, and comes to have natural stable beating rhythms even for $N \approx 10$. The universality of this community effect lies in the fluctuation dissipation theorem in statistical mechanics.

1 Introduction

Synchronization of biological cycles is indispensable to life activity [1, 2]. The heartbeat is the representative phenomenon of synchronization in physiology in which spontaneous pulsations of cardiomyocytes are tuned to a certain beating rate. Extensive work has been devoted to understanding the mechanism of regularity in beating of cardiac cells both experimentally and theoretically [3–9]. Contraction of a cardiomyocyte is caused by complex electrophysiological processes, and the detailed analyses require elaborated mathematical models composed of a huge number of equations [10,11]. To understand the essence of synchronization, however, a small number of simultaneous ordinary equations of membrane currents and action potentials, such as the Hodgkin-Huxley equation or its reduced form, the FitzHugh-Nagumo equation and the Van der Pol equation, are enough to capture the key phenomenon of the cell dynamics (see, for example, [12, 13]). Most

mathematical models for interacting cardiac cells are based on these equations [14–18]. Then a network of cardiomyocytes is regarded as a system of interacting self-sustained (nonlinear) oscillators. To explain the essence of synchronization in such a system of oscillators, phase equations have been used extensively and successfully [19, 20]. A variety of work with phase equations on a network of cardiac cells has been reported such as synchronization of cardiac pacemaker cells to the external periodic stimuli, phase resetting properties of cardiac cells [21], oscillation regularity depending on the cell networks [22], and so on.

Recently, an on-chip single-cell-based culture system has been developed. Small artificial networks of cardiomyocytes can be constructed and measured their spontaneous beating rhythms in terms of the effects of number of cells, configurations and kinds of cells [23, 24]. Although isolated cardiomyocytes are quite heterogeneous and their beating rhythms are inconsistent, even a pair of cardiomyocytes tend to synchronize when connected with each other. Since the features of an individual beating cell are now measurable and the configuration of a cellular network, which affects cell-to-cell interactions significantly, is flexibly constructible, it would be of considerable importance to examine how heterogeneity of cells and cell-to-cell interactions influence on synchronization in a small cluster of cardiomyocytes. There are two important observable quantities in a cardiomyocyte; One is its cell cycle (beating rate) and the other is its refractory period. In particular, a cardiomyocyte has quite a long refractory period comparing with that of a neuron, and the difference of the refractory periods among cardyomyocytes is expected to affect the behavior of synchronization. In case cardiomyocytes are isolated, they will just beat independently, while if they come into contact and interact with each other, their beating rhythms become synchronized. It was conjectured for a long time that, in a network of cardiomyocytes, firing of one cardiomyocyte triggers induced firing of the adjacent cardiomyocytes and all the cardiomyocytes start beating synchronously, and that the beating rate is tuned to the fastest one [5]. However, recent experiments have revealed that other cells are synchronized not to the fastest one but to the one with the least fluctuation of the beating rhythm [25].

The aim of the present article is to investigate the community effect of cardiomyocytes in different configurations of networks constituted by cells with specified characteristics of beating rhythms and to clarify how an assembly of cells acquires *stability*, one of the most important universal features in biological systems. Since it is quite difficult in a vitro experiment to prepare a cardiomyocyte with given properties, we develop a mathematical model which explains this behavior of cardiomyocytes with high reliability.

2 Summary of results in Chapter 2

Let us consider a simple system of two cardiomyocytes beating with regular cycles. We call the two cardiomyocytes cell-1 and cell-2 respectively. Each cardiomyocyte has its own cell cycle which coincides with the period of pulsation. In case cell-1 and cell-2 are isolated, they will just beat independently, while they are connected with each other, firing of cell-1 can influence cell-2 through the membrane potentials, and vice versa. Although a number of cell-to-cell interactions may take place when two cardiomyocytes come into contact, we discuss only the effect of firing of the adjacent cell.

We construct the phase equations for the dynamics of the two cardiomyocytes. The model is described by the phase variable $\phi_i(t)$ $(0 \leq \phi_i(t) \leq 2\pi,\ i = 1, 2)$ which denotes the state of cell-i at a time t and is defined over modulo 2π. We suppose that cell-i fires (beats) when $\phi_i(t) = 0\,(\equiv 2\pi)$. This firing takes place either $\phi_i(t)$ reaches 2π or the following conditions are satisfied; $\phi_i(t-0) \geq \theta_i$ and cell-j $(1 \leq j \neq i \leq 2)$ connected to cell-i fired retardation time τ ago, that is, $\phi_j(t-\tau) = 0$. Otherwise, that is, if cell-i is in the refractory period or cell-j does not fire, $\phi_i(t)$ is governed by the following differential equation: $d\phi_i(t) = \omega_i dt$. The equations for cell-i are given as

$$\begin{cases} d\phi_i(t) = \omega_i dt & (0 \leq \phi_i(t-0) \leq \theta_i \text{ or } \phi_j(t-\tau) \neq 0) \\ \phi_i(t) = 0 & (\theta_i \leq \phi_i(t-0) \leq 2\pi \text{ and } \phi_j(t-\tau) = 0) \end{cases} \tag{2.1}$$

where ω_i is an average phase velocity of the cell-i, θ_i is a phase corresponding to the refractory period of the cell-i $(0 < \theta_i < 2\pi)$, and τ is a delay time of signal propagation in adjacent cardiomyocytes.

We found that if cardiomyocytes have tight refractory periods, they eventually start synchronizing and their beating rate is equal to that of the faster beating cariomyocyte, which explains the postulations by Goshima. While, we found that if cardiomocytes have flexible refractory periods, under the assumption that the ratio of the refractory period to the firing period is the same in the two cardiomyocytes, their beating rhythms come to be synchronized or harmonized. Thus, modeling this system by the integrate and fire model with refractory period, we prove the postulations by Goshima for normal cells and get the phase diagram with complicated bifurcations of harmonization for abnormal cells. These phase diagrams indicate that cardiac arrhythmias can be caused by the transition of the phase.

3 Summary of results in Chapter 3

In chapter 2, we considered the model for two cardiomyoctes with regular cycles. The examination showed that a cardiomyocyte with lower beating fluctuation act as a pacemaker and the beating rhythm after synchronization is tuned to the stable cardiomyocyte [25]. It is difficult for this model to reproduce the experimental results because it does not take into account the biological noise. Here, we aim at a construction of a mathematical modeling reproducing the observation by extending the phase model(2.1).

Let us consider a network of two cardiomyocytes beating with irregular cycles. We propose an extended phase model which contains stochastic process and cell-to-cell interaction: then, the phase variable $\phi_i(t)$ is governed by the following interacting stochastic diffrential equation.

$$\begin{cases} d\phi_i(t) = \omega_i dt + dW(\sigma_i) + \sigma_i^2 V(\phi_i, \phi_j)dt & (0 \leq \phi_i(t-0) \leq \theta_i \text{ or } \phi_j(t-\tau) \neq 0) \\ \phi_i(t) = 0 & (\theta_i \leq \phi_i(t-0) \leq 2\pi \text{ and } \phi_j(t-\tau) = 0) \end{cases} \tag{3.1}$$

where $dW(\sigma)$ is a stochastic process with mean deviation σ, and $V(\phi_i, \phi_j)$ denotes the weak interaction through the membrane potential which we assume as the following form

$$V(\phi_i, \phi_j) := \mu \sin(\phi_j - \phi_i). \tag{3.2}$$

Here μ is a positive constant. Note that ω_i, θ_i and σ_i can be determined by single cell experiments for each cardiomyocyte. We utilize an extended random walk as the stochastic process $W(\sigma)$. The positive constant μ is the only parameter in our model which cannot directly measured by experiments. By defining an evaluation function, we are able to determine the free parameter μ to minimize the function.

In the experiments [25], the mean beating rate and its fluctuation before and after synchronisation were observed for 14 pairs of cardiomyocytes. We applied our model to determine whether it could reproduce the results of these pairs of cardiomyocytes. Except for pair No. 14, the simulated values accurately agree with the experimental values. Fluctuation in beating of a pair of synchronized cardiomyocytes almost coincided with that of less fluctuating cardiomyocytes, while the mean beating rate after synchronization was widely distributed. Some synchronized cardiomyocytes coincided with faster rates, some with slower rates, and others with intermediate rates. The experimental result of pair No.14 is exceptional because it is the only pair in which fluctuation increased after synchronization.

4 Summary of results in Chapter 4

We consider a network of N cardiomyocytes. Let k cells (cell-i_1, cell-i_2,...,cell-i_k) be connected to cell-i. Define S_i as the set of the index of cells connected to cell-i; $S_i := \{i_1, i_2, ..., i_k\}$. Similar to Chapter 3, the phase variable $\phi_i(t)$ is governed by the following stochastic differential equation.

$$\begin{cases} d\phi_i(t) = \omega_i dt + dW(\sigma_i) + \sigma_i^2 \sum_{j \in S_i} V(\phi_i, \phi_j) dt \ \ (0 \leq \phi_i(t-0) \leq \theta_i \text{ or } {}^{\forall} j \in S_i, \phi_j(t-\tau) \neq 0) \\ \phi_i(t) = 0 \qquad\qquad (\theta_i \leq \phi_i(t-0) \leq 2\pi \text{ and } {}^{\exists} j \in S_i, \phi_j(t-\tau) = 0) \end{cases} \tag{4.1}$$

where $V(\phi_i, \phi_j) := \mu \sin(\phi_j - \phi_i)$. As an application of our mathematical modeling, we then performed two numerical experiments on networks of cardiomyocytes and investigated the community effect of cadriomyocytes.

First, we investigated the dependence of fluctuation in beating rhythm of cardiomyocytes on the size and configuration of the system. The configurations that we considered were star, 2D lattice and 1D lattice networks. In all configurations, fluctuation rapidly decreased with an increase in the size of the system. Among the three configurations, a reduction in fluctuation was most rapid in the 2D lattice network, and fluctuation in the 1D lattice network was always larger than that in the other two configurations. For an ordinary stochastic ensemble, such as an independently identical distributed ensemble, the dependence of standard deviation of fluctuation on system size N was proportional to $N^{-1/2}$. However, the data of fluctuation considerably deviated from the line of $N^{-1/2}$ and the feature of beating fluctuation was relatively different from that of ordinary stochastic ensembles.

We then investigated the change in beating rhythms after connecting two subsystems of cardiomyocytes. we composed referential subsystems of four model cells and nine model cells so that these subsystems had the property of a standard beating rhythm (mean beating rate $1.20 \sim 1.30$ s; fluctuation $15.0 \sim 20.0$ [CV %]). As for the subsystems which are connected to referential subsystems, we considered subsystems consisting of four types of cardiomyocytes: (i) first and stable cell, (ii) first and unstable cell, (iii) slow and stable cell, and (iv) slow and unstable cell. For networks, we considered the three types of configurations; star, 2D lattice and 1D lattice networks. As a result, every cell started synchronizing after connection and fluctuation of the cells became equal in the combined system. Even a single stable cardiomyocyte could lower fluctuation of a network consisting of some cardiomyocytes.

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