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Regular Article

Observation of sarcomere chaos induced by changes in calcium concentration in cardiomyocytes

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Heating cardiomyocytes to 38–42°C induces hyperthermal sarcomeric oscillations (HSOs), which combine chaotic instability and homeostatic stability. These properties are likely important for achieving periodic and rapid ventricular expansion during the diastole phase of the heartbeat. Compared with spontaneous oscillatory contractions in cardiomyocytes, which are sarcomeric oscillations induced in the presence of a constant calcium concentration, we found that calcium concentration fluctuations cause chaotic instability during HSOs. We believe that the experimental fact that sarcomeres, autonomously oscillating, exhibit such instability due to the action of calcium concentration changes is important for understanding the physiological function of sarcomeres. Therefore, we have named this chaotic sarcomere instability that appears under conditions involving changes in calcium concentration as Sarcomere Chaos with Changes in Calcium Concentration (S4C). Interestingly, sarcomere instability that could be considered S4C has also been observed in the relaxation dynamics of EC coupling. Unlike ADP-SPOCs and Cell-SPOCs under constant calcium concentration conditions, fluctuations in oscillation amplitude indistinguishable from HSOs were observed. Additionally, like HSO, a positive Lyapunov exponent was measured. S4C is likely a crucial sarcomeric property supporting the rapid and flexible ventricular diastole with each heartbeat of the heart.

Key words: sarcomere chaos, hyperthermal sarcomeric oscillations, contraction rhythm homeostasis, excitation–contraction coupling

Significance

This study delves into the intricate relationship between hyperthermal sarcomeric oscillations (HSOs) caused by heating cardiomyocytes and calcium concentration fluctuations. It provides novel insights into how HSOs and calcium fluctuations impact the excitation-contraction coupling and the diastolic phase of the heart, introducing the term 'S4C' for the mechanism. This insight potentially paves the way for new possibilities in the treatment of cardiac diseases.

Introduction

The continuous and complex regulation of the heartbeat has traditionally been attributed to periodic changes in

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intracellular calcium concentrations within cardiomyocytes, dictated by the sinoatrial node [1–5]. Notwithstanding, the heart operates within a calcium concentration range that is not extreme, allowing the myocardium to neither fully contract nor relax, and thus ensuring constant ventricular blood filling during diastole [3–6].

Our recent research uncovered a hitherto unexplored facet of myocardial sarcomeres, which display rapid relaxation cycles despite high calcium levels [7–9]. We found that by warming cardiomyocytes to approximately body temperature, sarcomeres inside the cardiomyocytes undergo hyperthermal sarcomeric oscillations (HSOs), which involve a cyclic contraction and relaxation that closely follow the heartbeat and are independent of the electrical stimulation from the sinoatrial node [7–9]. Interestingly, contraction rhythm homeostasis maintains a regular cycle of HSOs despite sharp changes in amplitude and waveform caused by intracellular calcium concentration changes in different cycles [8]. Furthermore, we discovered that HSOs change their amplitude and phase chaotically to achieve both responsiveness and stability [9]. We hypothesized that the cyclic and rapid relaxation of the ventricular muscle during early diastole, even though the ventricular pressure is low and the intracardiomyocyte calcium concentration remains high, is a consequence of HSOs [8–10].

In this study, we investigated what kind of sarcomeric oscillations, including excitation–contraction coupling, underlie the chaotic instability observed in HSOs [11–16]. We first found that fluctuations in intracellular calcium concentration are important for the chaotic instability of HSOs. In addition, we found that chaotic instability is a common feature of HSOs before and during relaxation of excitation–contraction coupling. We believe that these observations are important for understanding the maintenance of a normal heartbeat and for diagnosis and fundamental treatment of heart disease resulting from a loss of maintenance [4–6,8,10,17,18].

Materials and Methods

Myofibril Preparation

The procedure for preparing myofibrils from rabbit psoas muscle was approved by the Institutional Animal Care and Use Ethical Committee of Waseda University and followed the Waseda University animal experimentation rules [16]. Experiments using myofibrils were approved by the Institutional Animal Care and Use Ethical Committees of Waseda University and the University of Tokyo [16]. Myofibrils were obtained from the intestinal psoas muscle of male Japanese White (JW) rabbits (2.5–3.0 kg, conventional) decapitated after anesthesia induced by injection of 25 mg/kg sodium pentobarbital into the ear vein. The intestinal psoas muscle was split into a thin bundle and immersed in a solution containing 50% v/v glycerol, 0.5 mM NaHCO3, and 5 mM EGTA (entire solution pH 7.6). Myofibrils were obtained by homogenizing the glycerinated muscle fibers in a solution containing 1 mM EGTA, 60 mM KCl, 5 mM MgCl, and 10 mM tris-maleate buffer (entire solution pH 7.5).

Cardiomyocyte Preparation

The isolation and use of myocardial cells followed the guidelines in the Animal Experiment Implementation Manual of the University of Tokyo and were approved by the Animal Experiment Committee of the Faculty of Science, The University of Tokyo [8]. Each batch of immature cardiomyocytes was harvested from ten one-day-old Wistar rats (Sankyo Labo Service Corporation, Tokyo, Japan) and cultured in a medium containing a mixture of Dulbecco's modified Eagle's medium and F-12 Nutrient Mixture (Life Technologies), 10% v/v fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin (Life Technologies). Ten neonatal rats used for one cardiomyocyte collection were born from the same female rat. The myocardial cell suspension collected is a mixture of 10 male and female rats. The cardiomyocytes were transfected with the pAcGFP-actinin plasmid [6–8] and observed one day later.

Observation of ADP-Induced Spontaneous Oscillatory Contractions (ADP-SPOCs) of Myofibrils

We used previous data [16] acquired as briefly described below. ADP-SPOCs were induced by exchanging the relaxing solution in which the myofibrils were immersed with the ADP-SPOC solution. ADP-SPOCs were observed at 25°C using phase-contrast optical microscopy at an imaging speed of 25 fps.

Induction and Observation of SPOCs in Cardiomyocytes (cell-SPOCs)

We used previous data [11] obtained as briefly described below. Cell-SPOCs were induced by immersing cardiomyocytes in a Ca-SPOC solution supplemented with 4 μM ionomycin (Sigma-Aldrich), 4 μM thapsigargin (Sigma-Aldrich), and 200 μM ryanodine (Sigma-Aldrich). Cell-SPOCs were observed using fluorescence microscopy at an imaging speed of 50 fps. The observation area of the glass-bottom dish in which the cardiomyocytes were cultured was maintained at 36°C during observation.

Induction and Observation of HSOs in Cardiomyocytes

We used previous data [8] acquired as briefly described below. An inverted microscope (IX-70, Olympus) was equipped

with a high-sensitivity camera (iXon 3 860, iXon Ultra, Andor Technology) and an oil-immersed objective lens (UPlanSapo 100 XO, Olympus). The pixel size of the camera corresponded to the cell length of 150 nm, and a frame rate of 500 fps was used for high-resolution temporal analysis. The temperature of the glass slide seeded with cardiomyocytes was maintained at (37.0 ± 0.2)°C using a thermostat-controlled incubator (INUG 2-ONICS, Tokai Hit) on the sample stage. A 1550 nm laser (FPL 1055 T, Thorlabs) was used as a heat source to rapidly change the temperature near the cardiomyocytes to be monitored. HSOs were induced by application of heat in the vicinity of cardiomyocytes. A 488 nm laser (FITEL HPU50211, Furukawa Electric Co., Ltd.) was used as an excitation light source for observing the fluorescence of AcGFP-α-actinin.

The observation of HSOs with simultaneous measurement of changes in Fluo4 fluorescence intensity used previous data [7]. In this observation, HSOs were observed in cases where there was a change in intracellular calcium concentration (HSOs with beating) and when the intracellular calcium concentration was maintained constant in the same manner as during Cell-SPOCs observation (HSOs without beating).

Observation of Spontaneous Beating of Cardiomyocytes

The spontaneous beating of cardiomyocytes before the above-described induction of HSOs was analyzed.

Measurement of Sarcomere Length (SL) Changes

As previously reported, the original intensity profiles for SL measurements were obtained using Solis software (Andor Technology) and analyzed using ImageJ (National Institutes of Health). SL was accurately measured by fitting the Z-line intensity profile to a parabolic function [7–9,11,16]. For ADP-SPOCs and cell-SPOCs, the oscillation amplitude of sarcomere length (SLA) was defined as the time series of SL changes minus the mean SL change. For HSOs, the time series of SL changes were bandpass-filtered (3.5 to 25 Hz) using LabChart 7 software (ADInstruments) to obtain the SLA. This filter removed the slow-fluctuating component derived from changes in intracellular calcium concentration, whose timescale is several times longer than that of HSOs, and enabled us to define the instantaneous phase of the HSO cycle by using additional signal processing described below. The same bandpass filter was applied to some spontaneous beat data for comparison with the HSO data.

Wave Pattern Analysis of Instantaneous Phase

The instantaneous phase of sarcomere oscillation was analyzed using the following previously developed method [9]. A Hilbert transform of the time-dependent SLA was performed using OriginPro 2022 software (OriginLab Corporation): $SLA_H(t) = \frac{1}{\pi} P.V. \int_{-\infty}^{\infty} \frac{SLA(\tau)}{t-\tau} d\tau$. Here, $P.V.$ represents integration in the sense of the Cauchy principal value. Then, the instantaneous amplitude $A(t) = \sqrt{SLA(t)^2 + SLA_H(t)^2}$ and instantaneous phase $P(t) = \mathrm{atan2}(SLA(t), SLA_H(t))$ were obtained, the range of $P\ (t)$ being $[-\pi, \pi]$ rad. $P(t)$ increased almost linearly from $-\pi$ to π and momentarily decreased from π to $-\pi$. We also obtained $P_S(t) = \sin(P(t))$ to make it easier to visualize the phase differences between sarcomeres that are spatially aligned.

Analysis of Chaotic Properties

The Lyapunov exponent was obtained as described previously [9]. First, we calculated the $SLA(t)$ trajectory in the three-dimensional phase space at time *t* and the difference between that value and the values 10 ms later ($SLA(t + \Delta\tau) - SLA(t)$) and 20 ms later ($SLA(t + 2\Delta\tau) - SLA(t)$). We then calculated the difference in Euclidean norm distance between two adjacent $SLA(t)$ orbits. If the natural logarithm of the orbital difference is linear in time, then the orbital difference increases exponentially. Performing linear regression in the 0–200 ms range, a fit with a corrected R^2 value of 0.8 or more was considered to be linear, and the slope was taken to be the Lyapunov exponent.

Variation in SL was analyzed for each time point as follows. First, the mean length of five consecutive sarcomeres was calculated. Then, the value of the mean squared error of each SL was calculated. Finally, the time series of the average of five mean squared error values was calculated.

Amplitude variation was analyzed for each time point as follows. Using the SLA maxima for each period of sarcomeric oscillation, the mean squared error between the average SLA maximum and the individual SLA maxima was calculated. The amplitude variation was taken to be the average mean squared error.

Statistics

All statistical tests were performed using a library of statistical software tools (https://www.r-project.org). The normality and homoscedasticity of the values used in principal component analysis were tested using Kolmogorov–Smirnov and Bartlett tests, respectively. Multiple comparisons between data that satisfied both normality and homoscedasticity assumptions were made using Dunnett's test. If the data did not satisfy the above conditions, Steel's multiple comparison test was used. Comparisons using Dunnett's and Steel's tests were made relative to the HSO group. The significance

threshold for all statistical analyses was 5%. The error bars in all figures indicate standard deviations.

Results

ADP-SPOCs in demembranated myofibrils are sarcomeric oscillations involving repeated contraction and relaxation of individual sarcomere repeats in a solution environment containing no calcium ions [13,15,16]. Adjacent sarcomeres continued to oscillate during ADP-SPOCs at slightly staggered amplitudes (Figure 1A). Because the timing deviation was almost constant, the Lissajous figure follows an elliptical orbit without any twist (Figure 1B). Cell-SPOCs in cardiomyocytes are sarcomeric oscillations induced by inhibitors of the sarcoplasmic reticulum's release and uptake of calcium and of ionophores that increase the calcium concentration-dependent permeability of the cell membrane [11]. When cell-SPOCs are induced, the intracellular calcium concentration is kept at a constant level that is undetectable using Fluo4, a calcium concentration indicator [11]. Neighboring sarcomeres in these cell-SPOCs also continued to oscillate with stable amplitudes and slightly staggered timing (Figure 1C). Again, since the timing deviation was almost constant, the Lissajous figure displays an elliptical orbit without any twist (Figure 1D). HSOs in living myocardial cells are sarcomeric oscillations induced by near-infrared heating of myocardial cells to 38–42°C [7–9]. During HSOs, the temperature of myocardial cells is close to core body temperature, and the EC coupling mechanism of myocardial cells is not inhibited. Therefore, by observing HSOs, we can understand how changes in calcium concentration associated with EC coupling affect the oscillation waveforms [7–9]. During HSOs, adjacent sarcomeres changed their oscillation amplitude in the cycle associated with the EC coupling, and they changed the timing of each other's oscillations (Figure 1E). The Lissajous figure shows a complicated trajectory (Figure 1F), although the oscillation period is stable, as can be seen from the waveform data (Figure 1E).

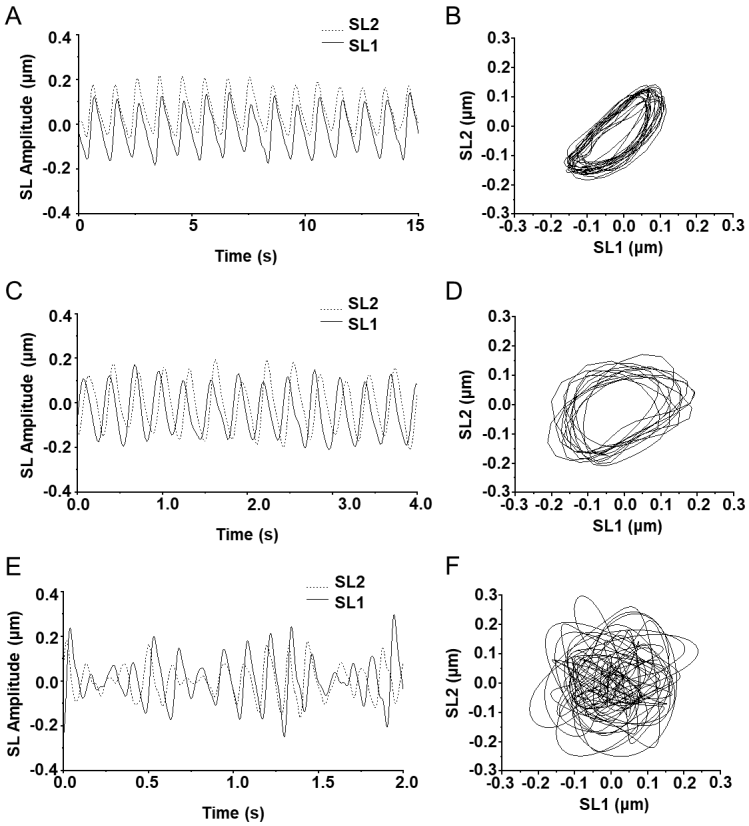

Figure 1 Characteristics of various sarcomeric oscillations. (A) Changes in amplitude of sarcomere length (SL) during ADP-induced spontaneous oscillatory contractions (ADP-SPOCs) of two adjacent sarcomeres in demembranated myofibrils. (B) Lissajous diagram of the two sarcomeres shown in panel A. Data acquired over a 20 s period are shown. (C) SL amplitude changes during cell-SPOCs of two adjacent sarcomeres in cardiomyocytes. (D) Lissajous diagram of the two sarcomeres shown in panel C. Data acquired over a 4 s period are shown. (E) SL amplitude changes during hyperthermal sarcomeric oscillations (HSOs) of two adjacent sarcomeres in living cardiomyocytes. (F) Lissajous diagram of the two sarcomeres shown in panel E. Data acquired over a 15 s period are shown.

Analysis of five successive sarcomeres showed that ADP-SPOCs and cell-SPOCs maintained a wave propagation pattern (Figure 2A, C). In contrast, the phases of adjacent sarcomeres switched irregularly between synchronous and antiphase states during HSOs (Figure 2E). Visualization of the instantaneous phase relationship between two adjacent sarcomeres also confirmed that waves propagated with a constant phase during ADP-SPOCs and cell-SPOCs (Figure 2B, D). However, during HSOs, we found that the phase relationship changed irregularly, resulting in a densely filled phase space (Figure 2F).

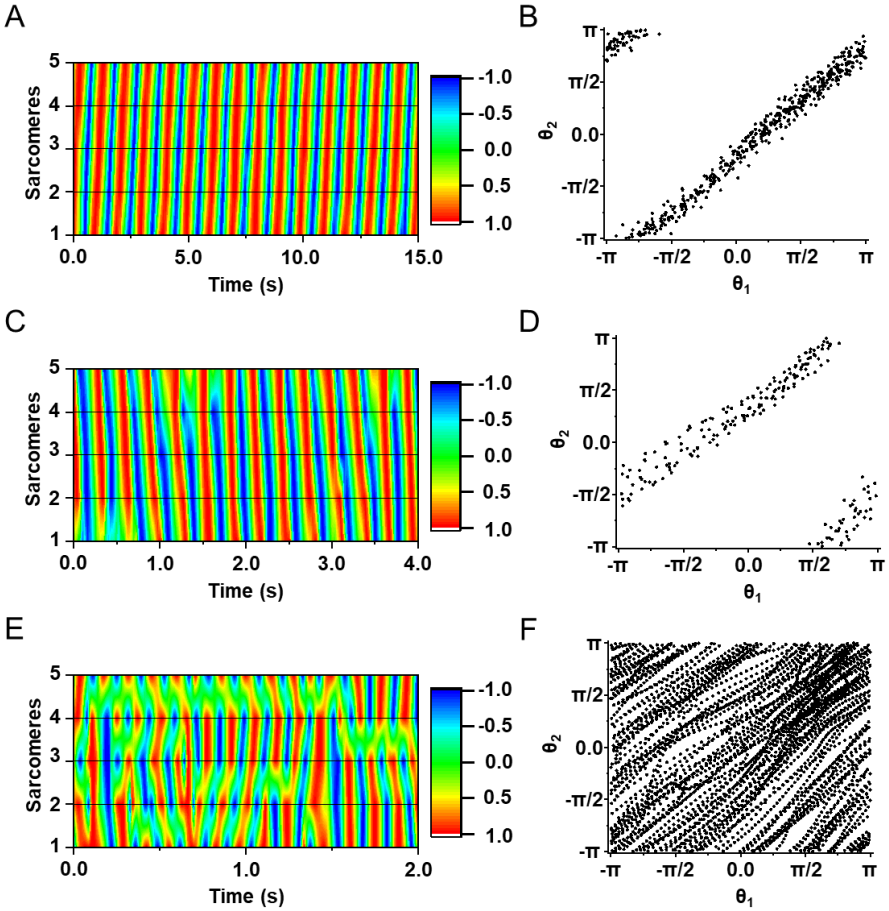

Figure 2 Wave pattern characteristics of sarcomeric oscillation. (A) Spatiotemporal distribution of the instantaneous phase (P_S (t)) of ADP-SPOCs in demembranated myofibrils. (B) Scatter plot of the instantaneous phase (P(t)) relationship of adjacent sarcomeres during ADP-SPOCs. Data acquired over a 20-s period are shown. (C) Spatiotemporal distribution of P_S (t) during cell-SPOCs in cardiomyocytes. (D) Scatter plot of the P(t) relationship of adjacent sarcomeres during cell-SPOCs. Data acquired over a 4-s period are shown. (E) Spatiotemporal distribution of P_S (t) during HSOs in living cardiomyocytes. (F) Scatter plot of the P(t) relationship of adjacent sarcomeres during HSOs. Data acquired over a 5-s period are shown.

In order to compare the effects of the presence or absence of changes in calcium concentration more strictly, we simultaneously measured the changes in intracellular calcium concentration under two conditions: one where the intracellular calcium concentration remains constant, referred to as HSOs without beating, and another where the intracellular calcium concentration fluctuates, referred to as HSOs with beating. The results showed that in HSOs without beating, the oscillation amplitude was stable, and the phase relationship of adjacent sarcomeres maintained a slight shift in timing. Consequently, the Lissajous figures formed non-twisted elliptical orbits (Figure 3A, B). In contrast, in HSOs with beating, the oscillation amplitude varied, and the phase relationship of adjacent sarcomeres frequently changed to in-phase or anti-phase (Figure 3C). As a result, the Lissajous figures became complex trajectories (Figure 3D). Even when capturing the wave pattern of five consecutive sarcomeres, it was confirmed that HSOs without beating maintained the propagating wave pattern, whereas HSOs with beating frequently switched to in-phase or anti-phase (Figure 3E). At the same time, using cardiomyocytes obtained from the same neonatal rats, and evoked by the same infrared laser irradiation-

induced heating, this difference was observed in HSOs [7]. From these observations, it was clearly confirmed that under conditions involving fluctuations in calcium concentration in HSOs, chaotic sarcomere instability appears (Figure 3). The 'chaotic instability' mentioned here refers to instability that follows certain rules, affecting aspects of sarcomere oscillation other than the period, such as 'amplitude' and 'velocity,' changing according to the properties of physical chaos, while maintaining a constant 'period'. We believe that this experimental fact, that sarcomeres capable of autonomously oscillating and forming wave patterns exhibit such instability due to changes in calcium concentration, is important in understanding the physiological functions produced by sarcomeres. Therefore, we have named this chaotic sarcomere instability that appears under conditions involving changes in calcium concentration as Sarcomere Chaos with Changes in Calcium Concentration (S4C).

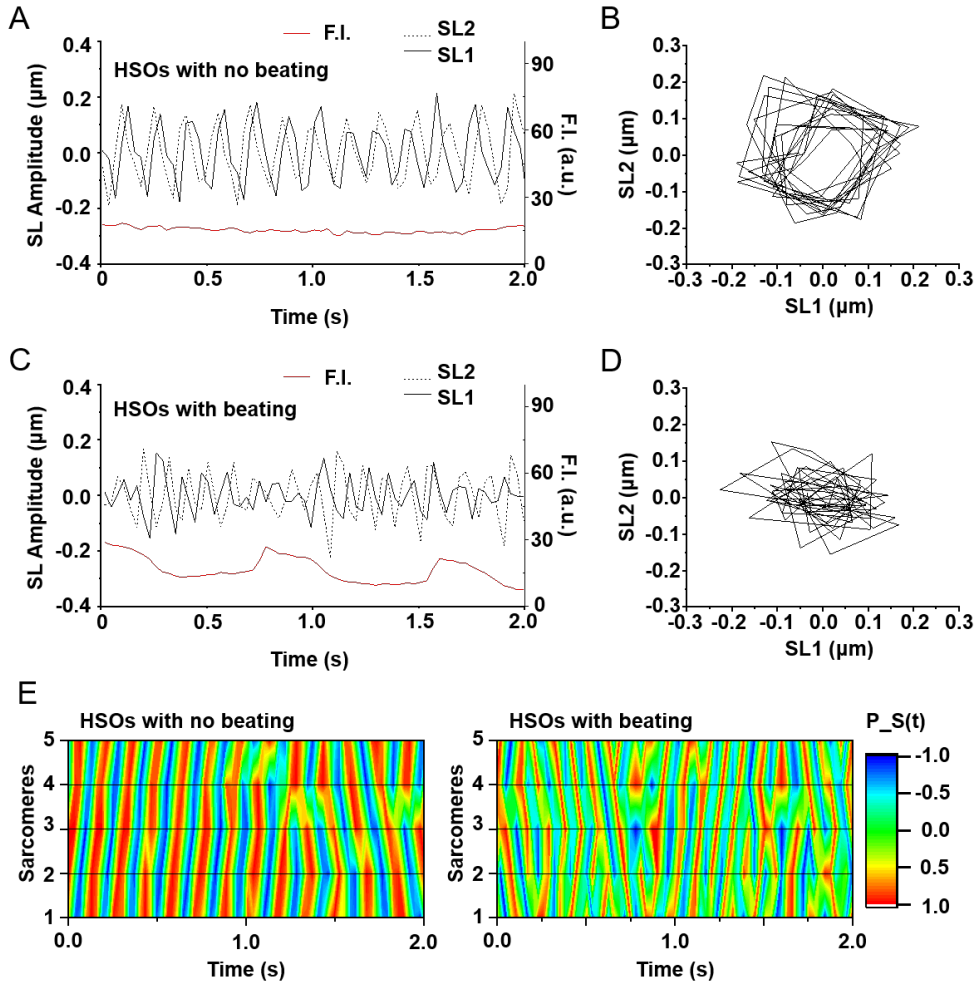

Figure 3 Differences in HSOs due to the presence or absence of intracellular calcium concentration changes. (A) The change in SL amplitude of two adjacent sarcomeres during HSOs without beating with constant intracellular calcium concentration. The red line indicates changes in Fluo4 fluorescence intensity, reflecting changes in intracellular calcium concentration. (B) Lissajous diagram of the two sarcomeres in (A). (C) The change in SL amplitude of two adjacent sarcomeres during HSOs with beating when intracellular calcium concentration fluctuates. The red line is the same as in (A) indicating changes in Fluo4 fluorescence intensity, reflecting changes in intracellular calcium concentration. (D) Lissajous diagram of the two sarcomeres in (C). (E) Spatiotemporal distribution of P_S (t) in HSOs in living cardiac muscle cells. The left image is during HSOs without beating, and the right image is during HSOs with beating.

The S4C of HSOs creates contraction rhythm homeostasis by maintaining a constant oscillation period while responding to waveform changes due to fluctuations in intracellular calcium concentration. Therefore, it is considered a type of instability associated with order. Then, in the sarcomere dynamics of EC coupling, does this sarcomere instability, referred to as S4C, occur? Before inducing HSOs by irradiating with an infrared laser, we were able to observe the cell's spontaneous EC coupling. We analyzed the stability of sarcomere dynamics associated with this EC coupling. It was found that even during EC coupling before HSO induction by heating, the behavior of individual sarcomeres changes

during relaxation (Figure 4A–C). The phase instability was also confirmed by examining the instantaneous phases of adjacent sarcomeres (Figure 4D). ADP-SPOCs and cell-SPOCs were characterized by minor oscillation amplitude fluctuations, but EC coupling and HSOs produced large amplitude fluctuations that were statistically indistinguishable (Figure 4E). Therefore, the Lyapunov exponent was also measured for EC coupling and found to have an average value of 8.96 ± 1.27 (n = 21). These results suggest that the relaxation dynamics of EC coupling may indicate a potential for sarcomere instability, which can be referred to as S4C (Figure 4).

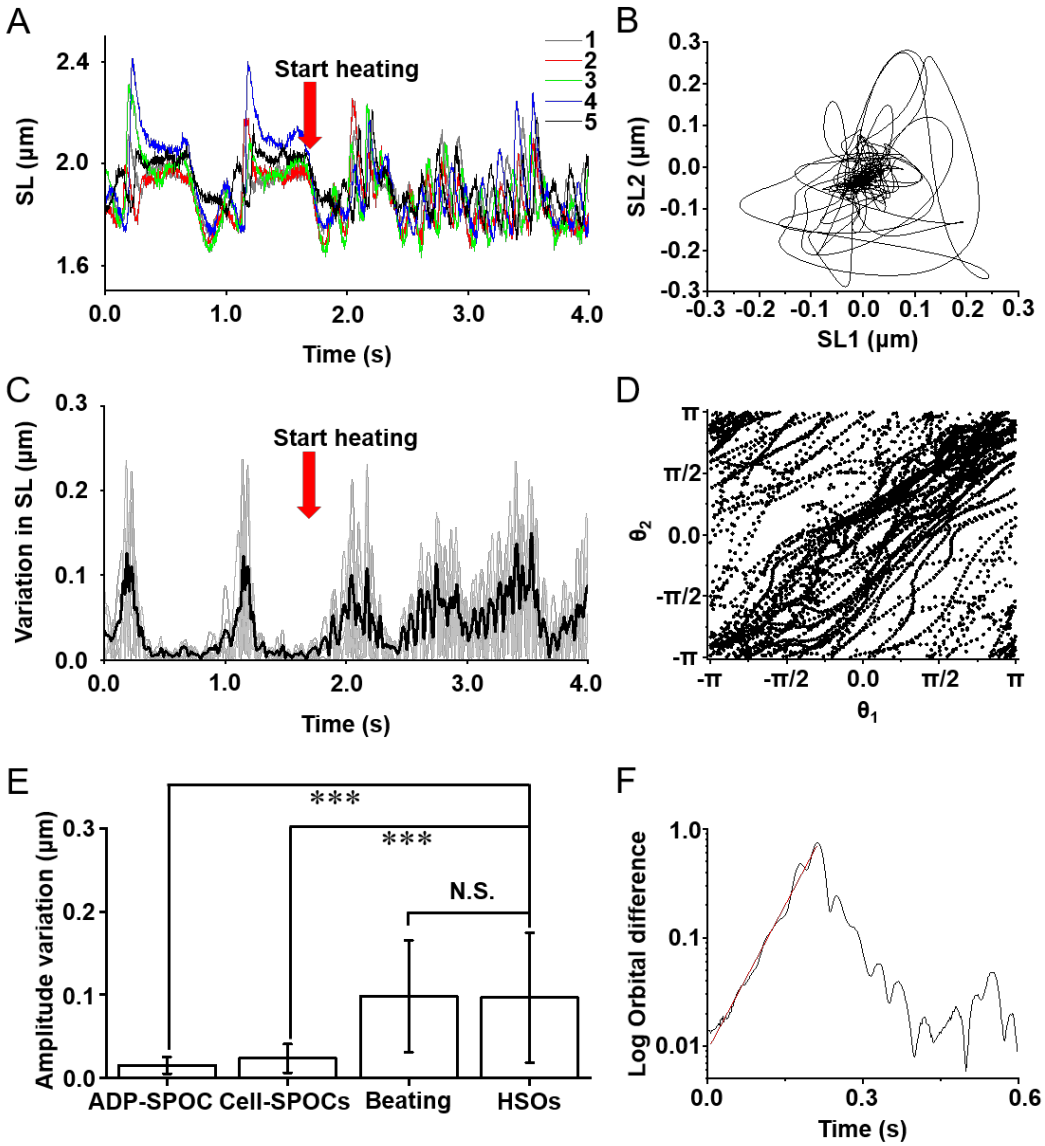

Figure 4 Chaotic properties common to excitation–contraction coupling and HSOs. (A) Temporal changes in SL of five adjacent sarcomeres in cardiomyocytes. Heat was applied to the cells at the time indicated by the red arrow to initiate HSOs. (B) Lissajous diagram of the third and fourth sarcomeres shown in panel A. Data acquired over a 9-s period are displayed. (C) Analysis of SL variation. Temporal changes in the mean value of five consecutive SLs (black line) and the magnitude of individual SL differences (gray lines) are shown. (D) Scatter plot of P (t) for adjacent sarcomeres during EC coupling. Data acquired over a 9 s period are shown. (E) Amplitude variation during each sarcomeric oscillation. Beating is the spontaneous dynamics due to EC coupling observed before heating to induce HSOs. Error bars are standard deviation. (F) Changes in the Euclidean norm distance between two trajectories of SL oscillation amplitude during the relaxation step of EC coupling. The red line shows the result of linear regression performed in the 0–200 ms range and has a slope equal to the Lyapunov exponent. The vertical axis plots the common logarithm.

Discussion

We have observed autonomous sarcomeric oscillations under various conditions to date [7–9,11,16]. We have also improved our technical skills in analyzing sarcomeric oscillations, such as defining the instantaneous phase and visualizing disrupted waveforms as part of the analysis [9,19]. In this study, we conducted a comparative investigation analyzing these various autonomous oscillations with the latest analytical methods (Figure 1–3). The results clearly re-demonstrated that HSOs continuing autonomous sarcomeric oscillations under conditions affected by changes in intracellular calcium concentration are highly unique oscillations (Figure 1–3). In sarcomeric oscillations such as ADP-SPOCs and Cell-SPOCs that occur at a constant calcium concentration, a stable wave of propagating oscillations was created, forming elliptical orbits in the Lissajous figures produced by the oscillation waveforms of adjacent sarcomeres (Figure 1, 2). However, HSOs showed stability by altering the oscillation amplitude in response to calcium concentration changes while maintaining a constant oscillation period, yet they also demonstrated chaotic instability with complex trajectories in the Lissajous figures formed by the oscillation waveforms of adjacent sarcomeres (Figure 1, 2). At the same time, using cardiomyocytes obtained from the same neonatal rats and evoked by the same infrared laser irradiation-induced heating, we observed similar differences in HSOs (Figure 3). Firstly, under HSOs without beating conditions where the intracellular calcium concentration remains constant, the Lissajous figures formed by the oscillation waveforms of adjacent sarcomeres became elliptical orbits (Figure 3A, B). On the other hand, under HSOs with beating conditions where the intracellular calcium concentration fluctuates, the Lissajous figures became complex trajectories (Figure 3C, D). Even when visualizing the waveforms, the HSOs without beating maintained a pattern of propagating waves, whereas the HSOs with beating frequently switched phases to in-phase or anti-phase (Figure 3E).

Therefore, we have named this chaotic sarcomere instability that appears under conditions involving changes in calcium concentration as Sarcomere Chaos with Changes in Calcium Concentration (S4C).

S4C likely holds significant potential for understanding the physiological functions produced by sarcomeres. Indeed, HSOs demonstrating S4C respond sharply to slow changes in intracellular calcium concentration, altering oscillation waveforms and amplitudes while maintaining a constant oscillation period [8,9]. We have named this property 'contraction rhythm homeostasis' [8]. We believe that in a series of sarcomeres sharing tension, this characteristic of maintaining a constant oscillation period can be explained as follows: even if the total number of myosin molecules generating force within a sarcomere increases with changes in calcium concentration, the force on individual myosin molecules remains constant, thus the oscillation period does not change [8]. This explains how the properties of S4C, which flexibly shifts the phase relationship between adjacent sarcomeres to in-phase or anti-phase, enable the total tension generated by a group of sarcomeres to smoothly transition independent of individual sarcomere oscillation states [9]. Also, it is easier for a disrupted wave pattern to further disintegrate than for an orderly wave pattern to break down, which accounts for the sensitivity to sharp changes [9]. Thus, it is strongly suggested that S4C is intimately involved in the mechanism that enables HSOs to respond sharply to changes while maintaining stability with a constant period.

S4C may also manifest in the relaxation dynamics of EC coupling. The autonomous oscillation characteristics of sarcomeres become evident at somewhat higher calcium concentrations than those of relaxation conditions [7,11–15]. In cardiomyocytes heated to 37°C, conditions are created where the calcium concentration remains high and the sarcomeres are still contracting following the onset of contraction dynamics of EC coupling [11]. We hypothesize that here, the autonomous sarcomeric oscillation characteristics become evident, actively forming propagating waves as part of the relaxation dynamics [4,10,11]. Furthermore, autonomous sarcomere dynamics occur in a fluctuating calcium environment, leading to dynamics associated with sarcomere instability that can be termed 'EC coupling S4C' (Figure 4). Indeed, unlike ADP-SPOCs or Cell-SPOCs under constant calcium conditions, fluctuations in oscillation amplitude indistinguishable from those in HSOs have been observed (Figure 4E). Furthermore, it has also become clear that the relaxation dynamics of EC coupling, similar to HSO, can be characterized by a measurable positive Lyapunov exponent (Figure 4F). Therefore, we highly suspect that the sarcomere instability in these relaxation dynamics of EC coupling is a characteristic that could be termed 'EC coupling S4C'.

S4C may be important for the flexible, sharp, and rapid relaxation of ventricular muscle during the early diastole, despite low ventricular pressure and high intracellular calcium concentration in the heart cells.

Adding to our findings, our most recent observations through real-time scanning electron microscopy of isolated mouse hearts submerged in culture medium have also verified the presence of complex relaxation oscillations [20]. These new insights emphasize that the mechanical properties of the heart should be viewed as continuous dynamics, providing a more nuanced understanding of cardiac function. This perspective underscores the importance of ongoing research into the inherent complexities of cardiac mechanics and the potential implications these complexities could have on our understanding and treatment of cardiac diseases.

Conclusion

Heating cardiomyocytes to 38–42°C triggers hyperthermal sarcomeric oscillations (HSOs) with a unique blend of chaotic instability and homeostatic stability, potentially contributing to rapid ventricular expansion during the diastole phase of the heartbeat. Fluctuating calcium concentrations, we found, contribute to the chaotic instability observed during HSOs. It has become clear that there is a significant correlation between the relaxation dynamics of HSOs and EC coupling, characterized by high fluctuation amplitude and a positive Lyapunov exponent. We introduced 'S4C' to describe this instability of sarcomere dynamics due to calcium fluctuations during the relaxation phase of excitation-contraction coupling and during HSOs. This research sheds light on complex cardiac dynamics and could potentially enhance our understanding of cardiac function and disorders.

Conflict of Interest

The author declare no competing interests.

Author Contributions

S.A.S. designed the project, conducted the experiment, analyzed the experimental data, and wrote the paper.

Data Availability

The evidence data analyzed during the current study are available from the corresponding author on reasonable request.

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