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Biophysical Chemistry 104 (2003) 509–522

Biophysical Chemistry

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Sensitivity and flexibility of regular and chaotic calcium oscillations

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Received 6 December 2002; received in revised form 12 February 2003; accepted 13 February 2003

Abstract

Sensitivity and flexibility are important properties of biological systems. These properties are here investigated for intracellular calcium oscillations. For a particular model, we comparatively investigate sensitivity and flexibility of regular and chaotic Ca^{2+} oscillations. For this model, we obtain two main results. First, sensitivity of the model system to parameter shifting does not depend on the complexity of Ca^{2+} oscillations. We observe, however, that both regular and chaotic Ca^{2+} oscillations are highly sensitive in regions close to bifurcation points. Second, also flexibility of Ca^{2+} oscillations does not significantly depend on the type of Ca^{2+} oscillations. Our results show that regular as well as chaotic Ca^{2+} oscillations in the studied model are highly flexible in regimes with weak dissipation. Both results are discussed in the sense of possible biological importance.

Keywords: Calcium oscillations; Chaos; Periodic forcing; Lyapunov exponents; Dissipation; Bifurcation

1. Introduction

The importance of cytosolic calcium is well established in a large variety of cell types. In excitable as well as in non-excitable cells, a significant part of signal transduction from receptors at the cell membrane to enzymes, controlling the complex behaviour of the biological systems, is performed by the oscillatory changing in free cytosolic Ca^{2+} concentration, the so-called Ca^{2+} oscillations. They regulate many cellular processes from egg fertilisation to cell death [1]. The mechanisms of these oscillations have been intensely investigated both from experimental and theoretical point of view (for review see [2,3]).

Calcium has to play a multiplicity of roles in order to trigger different cellular functions [1]. Therefore, flexible, yet precisely regulated, information encoding of Ca^{2+} oscillations in their frequency [4–10] as well as in their amplitude [11,12] is required. Thus for reliable functioning, a biological system has to be stable, highly sensitive and flexible. Suguna et al. [13] showed that a minimal condition for a model system to be stable, highly sensitive and flexible is a combination of a pair of coupled negative and positive feedback processes. However, the question remains under what conditions, determined by the model parameters, the system is most sensitive and flexible.

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doi:10.1016/S0301-4622(03)00038-3

Some authors have already dealt with similar questions. Kummer et al. [14], Läer et al. [15] and Zhong et al. [16] have investigated the impact of noise on the sensitivity of Ca^{2+} oscillations. It has been shown that adding Gaussian noise to a subthreshold extracellular stimulus causes noise-induced Ca^{2+} oscillations. This means that noise increases the sensitivity in the dose-response relation of calcium, which may have important physiological consequences in enhancing the detection of weak input signals. Galvanovskis and Sandblom [17,18] also studied the sensitivity of Ca^{2+} oscillations in response to weak external forcing and noise. Their experimental results showed influences of low frequency electromagnetic fields on the behaviour of living systems. They also studied this phenomenon theoretically on two mathematical models of intracellular Ca^{2+} oscillations: the two-variable model of Goldbeter et al. [19] and the three-variable model proposed by Shen and Larter [20]. In the latter, the sensitivity of regular and chaotic regimes was compared. Results indicated higher sensitivity of chaotic Ca^{2+} oscillations to variation of parameter values, suggesting a possible role of chaotic processes in detection of weak signals within cells.

The aim of the present study is to analyse the interrelation between sensitivity and flexibility of the same model system as studied by Sandblom and Galvanovskis (i.e. the model proposed by Shen and Larter [20]) and to determine the conditions under which the model is extremely sensitive and flexible. In particular, sensitivity and flexibility of regular and chaotic regimes are compared. Sensitivity is quantified by changes in the model variables caused by shifting the parameter values. We take use of the so-called response coefficients, which were defined in the theoretical framework of the metabolic control analysis (see e.g. [21]) and afterwards successfully applied to the control analysis of different periodic phenomena in biological systems (see e.g. [22,23]). For better comparison of our results to that obtained earlier by Galvanovskis and Sandblom [17] for the same model system, we use a slightly modified definition of the response coefficients. We shift the parameter values of the model system and calculate the ratio of the power of modulated Ca^{2+} oscillations over the power of non-modulated Ca^{2+} oscillations. This gives information about the energy change of the signal related mainly to its amplitude, however, not so much to its frequency. Therefore, we additionally examine the sensitivity of the system regarding changes in the oscillation frequency, by shifting the parameter values and calculating the ratio of the dominant oscillation frequency of modulated Ca^{2+} oscillations over the dominant oscillation frequency of non-modulated Ca^{2+} oscillations. Flexibility of the model system is studied by applying external periodic forcing to the model and analysing the changes in frequency of Ca^{2+} oscillations. The span of the frequency range in which Ca^{2+} oscillations are synchronised with the forcing signal represents the system ability to adapt the basic Ca^{2+} oscillations to the external forcing signal. Thus, the span of the frequency range is taken as a measure for the flexibility of the system.

2. Mathematical model

We analyse sensitivity and flexibility of the mathematical model proposed by Shen and Larter [20]. The functioning of the model system is based on the mechanisms of calcium-induced calcium release (CICR) [24] and the inositol trisphosphate crosscoupling (ICC) [25,26]. The ICC and CICR mechanisms provide two positive feedbacks (for details see [20]). There are three variables in the model: free Ca^{2+} concentration in the cytosol (Ca_{cyt}), free Ca^{2+} concentration in the ER (Ca_{er}), and the inositol trisphosphate concentration in the cytosol (IP_3). The evolution of the model system is governed by the following differential equations (for parameter values see Table 1):

$$\frac{dCa_{cyt}}{dt} = J_{ch} + J_{leak} - J_{pump} + J_{in} - J_{out}, \quad (1)$$

$$\frac{dCa_{er}}{dt} = J_{pump} - J_{ch} - J_{leak}, \quad (2)$$

$$\frac{dIP_3}{dt} = J_{+} - J_{-}, \quad (3)$$

Table 1
Model parameters for which all results are calculated unless otherwise stated

Parameter	Meaning	Value
k_{ch}	Maximal rate constant of Ca^{2+} channels in the ER membrane	3000.0 $\mu M\ s^{-1}$
k_{leak}	Rate constant of Ca^{2+} leak flux through the ER membrane	1.0 s^{-1}
k_{pump}	Rate constant of ATP-ases in the ER membrane	50.0 $\mu M\ s^{-1}$
k_{in1}	Rate constant for the agonist-depended influx into the cell	4.0 $\mu M\ s^{-1}$
k_{in2}	Constant Ca^{2+} influx into the cell	1.0 $\mu M\ s^{-1}$
k_{out}	Rate constant for Ca^{2+} efflux from the cell	10.0 s^{-1}
K_1	Half-saturation constant for the IP_3 binding to the Ca^{2+} channel	0.2 μM
K_2	Threshold constant for Ca^{2+} pumping into the ER	0.2 μM
K_3	Dissociation constant of the Ca^{2+} dependent component of PLC	1.0 μM
K_4	Activation constant of CICR	0.69 μM
K_5	Inhibition constant of CICR	0.69 μM
k_+	Maximal rate constant of IP_3 production	4.0 $\mu M\ s^{-1}$
k_-	Rate constant for IP_3 degradation by 5-phosphomonoesterase	2.0 s^{-1}
r	Degree of cell stimulation by agonist	0–1.0
a	Amplitude of periodic forcing	0.08–0.25 $\mu M\ s^{-1}$

where,

$$J_{ch} = k_{ch}\left(\frac{IP_3^4}{IP_3^4 + K_1^4}\right) \times \left(\frac{K_4 Ca_{cyt}}{(Ca_{cyt} + K_4)(Ca_{cyt} + K_5)}\right)^3 Ca_{er}, \quad (4)$$

$$J_{leak} = k_{leak} Ca_{er}, \quad (5)$$

$$J_{pump} = k_{pump}\frac{Ca_{cyt}^2}{Ca_{cyt}^2 + K_2^2}, \quad (6)$$

$$J_{in} = k_{in1} \cdot r + k_{in2} \quad (7)$$

$$J_{out} = k_{out} Ca_{cyt}, \quad (8)$$

$$J_+ = k_+ \cdot r\frac{Ca_{cyt}}{Ca_{cyt} + K_3}, \quad (9)$$

$$J_- = k_- \cdot IP_3 \quad (10)$$

The model exhibits both simple and complex Ca^{2+} oscillations and hence it is suitable to be used for a comparative study of how regular and chaotic Ca^{2+} oscillations are sensitive to parameter changes and how they are flexible in response to external periodic forcing.

For the external periodic forcing a simple sinusoidal function $f(t)$ is taken:

$$f(t) = a\ \sin(2\pi\nu_f t) \quad (11)$$

where a is the amplitude and ν_f is the frequency of the external forcing. The periodic forcing is considered as a variable Ca^{2+} flux across the cell membrane. In the model this is realised by inclusion of the $f(t)$ as additional term in Eq. (1).

All results are calculated for the parameter values given in Table 1 if not otherwise stated.

3. Results

The basic model system without periodic forcing expresses regular and chaotic Ca^{2+} oscillations. For better insight into the system behaviour, we calculate the largest Lyapunov exponent λ_{max}, which determines if the oscillatory regime is regular or chaotic. The largest Lyapunov exponent is calculated with the algorithm proposed by Wolf et al. [27]. In Fig. 1 the results are plotted vs. parameter r, which corresponds to the level of cell stimulation. The positive values of the largest Lyapunov exponent in Fig. 1 indicate that the model system behaves chaotically in two narrow parametric intervals $0.6175 < r < 0.6205$ and $0.6245 < r < 0.6275$. For other parameter values in the oscillatory regime between the two subcritical Hopf bifurcations at $r = 0.2345$ (HB1 in Fig. 1) and $r = 0.6859$ (HB2 in Fig. 1), the system expresses regular periodic oscillations. Both bifur-

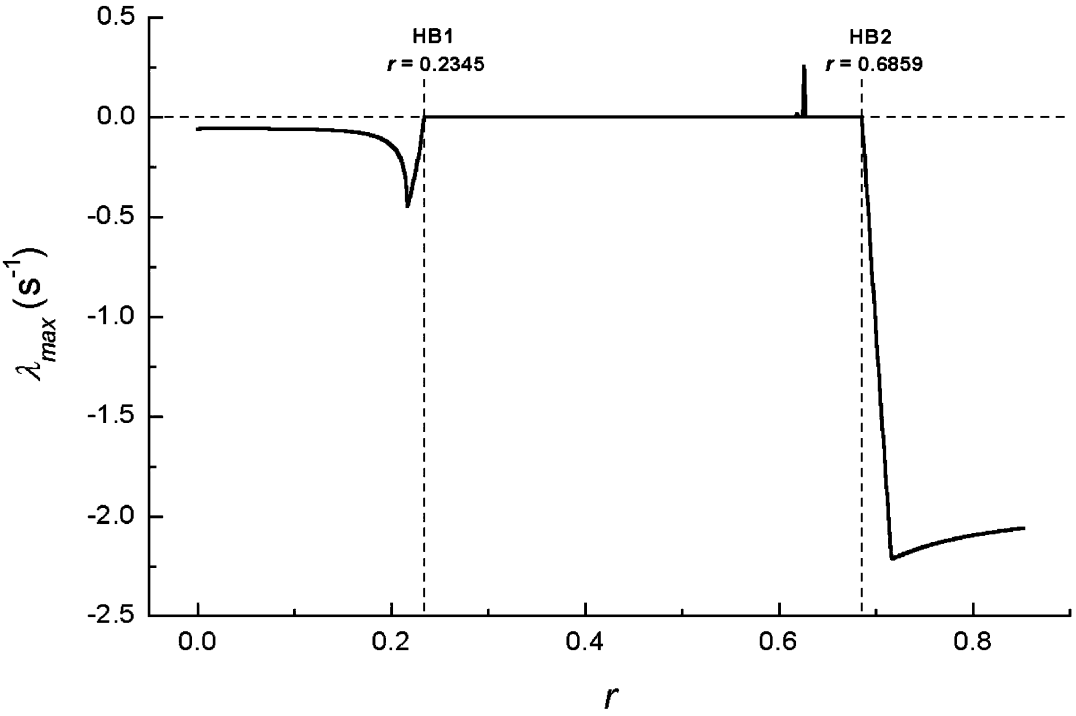

Fig. 1. The largest Lyapunov exponent λ_{max} is plotted vs. parameter r.

cation points were calculated by the program XPPAUT [28].

To examine the sensitivity of Ca^{2+} oscillations in the mathematical model, we shift the parameter values given in Table 1 by $\pm 10\%$ each at a time, and calculate the corresponding changes in model variables. To quantify changes resulting from the particular parameter shift, we take use of the response coefficients (R_p^X), defined by the following equation (e.g. [21–23]):

$$R_p^X = \frac{\partial X/X}{\partial p/p} = \frac{p}{X} \cdot \frac{\partial X}{\partial p}, \tag{12}$$

where p is a system parameter and X is a dependent variable, like for example ionic concentrations or any of the elements of the Fourier spectrum. For better comparison of our results with that obtained previously by Galvanovskis and Sandblom [17], we take a simplified definition for the sensitivity. Since in all cases the parameters are changed for the same percent (i.e. $\pm 10\%$), it holds $\partial p/p =$ konst., and the system sensitivity (S_X) can be simply estimated by the ratio (see also [17]):

$$S_X = \frac{X_{\text{mod}}}{X_{\text{non-mod}}}, \tag{13}$$

where X_{mod} and $X_{\text{non-mod}}$ are values of a particular system variable after (modulated) and before (non-modulated) the parameter shift, respectively. High sensitivity corresponds to ratios $S_X \ll 1$ and $S_X \gg 1$.

First, the system sensitivity regarding changes in the power of Ca^{2+} oscillations is analysed [$X = P$ in Eq. (13)]. We shift the parameter values given in Table 1 by $\pm 10\%$ each at a time, and calculate the corresponding ratios of the power of modulated oscillations over the power of non-modulated oscillations (S_P). The results are presented in Table 2a. It should be noted that the power of Ca^{2+} oscillations is calculated for the oscillating signal of Ca_{cyt} with the subtracted average value of the signal. Therefore, if the ratio of the power of modulated oscillations over the

Table 2
Effects of shifting the parameter values by ±10% for three different levels of cell stimulation: $r=0.50$, $r=0.62$ and $r=0.68$

Modulated parameter	Regular regime at $r=0.50$		Chaotic regime at $r=0.62$		Regular regime at $r=0.68$	
	−10%	+10%	−10%	+10%	−10%	+10%
(a)						
k_{ch}	0.99	1.02	1.29	0.42	1.22	0
k_{leak}	1.07	0.94	1.36	0.65	1.09	0.89
k_{pump}	0.93	1.08	0.32	1.49	0	1.43
k_{in1}	0.93	1.08	1.25	0.45	1.39	0
k_{in2}	0.96	1.05	1.25	0.51	1.17	0
k_{out}	1.16	0.87	0	1.19	0	1.26
K_1	0.93	1.05	0.36	1.52	0	1.23
K_2	1.17	0.83	1.73	0.02	2.44	0
K_3	0.95	1.05	0.47	1.44	0.72	1.16
K_4	0.91	1.08	0.61	1.34	0	1.35
K_5	0.95	1.03	0	1.45	0	1.93
r	0.97	0.93	1.49	0.27	4.96	0
(b)						
k_{ch}	0.97	1.02	1.21	5.10	0.93	0
k_{leak}	0.94	1.05	1.19	0.78	0.97	1.02
k_{pump}	1.09	0.93	5.75	1.11	0	0.90
k_{in1}	0.93	1.07	1.13	5.80	0.86	0
k_{in2}	0.97	1.03	1.21	5.03	0.97	0
k_{out}	1.02	0.97	0	1.21	0	0.98
K_1	1.12	0.89	6.11	1.08	0	0.86
K_2	0.90	1.10	1.02	10.1	0.67	0
K_3	1.12	0.91	5.70	1.10	1.11	0.90
K_4	0.97	1.02	4.52	1.32	0	1.04
K_5	1.05	0.95	0	1.1	0	0.78
r	0.81	1.19	0.95	6.66	0.18	0

Results are expressed (a) as ratios of the power of modulated Ca^{2+} oscillations over the power of non-modulated Ca^{2+} oscillations (S_P), (b) as ratios of the dominant oscillation frequency of modulated Ca^{2+} oscillations over the dominant oscillation frequency of non-modulated Ca^{2+} oscillations (S_ν).

power of non-modulated oscillations equals zero, this means that the system has ceased oscillating altogether. In Table 2a the results are presented for parameter values $r=0.50$, $r=0.62$ and $r=0.68$. For parameter values $r=0.50$ and $r=0.62$ the results are fully in agreement with those obtained by Galvanovskis and Sandblom [17], showing that the chaotic regime at $r=0.62$ is more sensitive than the regular one at $r=0.50$. On the other hand, however, the very high sensitivity of regular periodic state at $r=0.68$ shows that in general chaotic states are not more sensitive than regular states. We further tested the sensitivity for another periodic regime at $r=0.24$. We found that this regular periodic regime is also more sensitive than the chaotic regime at $r=0.62$ (data not shown). Thus, our results indicate that the system sensitivity does not necessarily depend on the complexity of Ca^{2+} oscillations.

To confirm these results even further we also analyse the sensitivity of Ca^{2+} oscillations regarding changes in the oscillation frequency [$X=\nu$ in Eq. (13)]. We shift the parameter values given in Table 1 by ±10% each at a time again, and calculate the corresponding ratios of the frequency of modulated oscillations over the frequency of

non-modulated oscillations (S_ν). The results, showing the sensitivity of Ca^{2+} oscillations regarding changes in the oscillation frequency, are presented in Table 2b and are fully in agreement with the results presented in Table 2a. High sensitivity corresponds to values $S_\nu \ll 1$ and $S_\nu \gg 1$ whereas $S_\nu = 0$ means that the system has ceased oscillating altogether. By comparing the results in Table 2a,b with Fig. 1 we see that regimes of high sensitivity coincide with the proximity of Hopf bifurcations, regardless of the complexity of Ca^{2+} oscillations.

Since our results show that chaos doesn't necessarily imply higher sensitivity of the system, it remains of interest to investigate the flexibility of chaotic and regular periodic regimes. Intuitively, chaos should be more flexible in response to external forcing since it is characterised by a variety of different amplitudes and non-harmonic frequencies. In order to examine the flexibility of Ca^{2+} oscillations in the mathematical model proposed by Shen and Larter [20] we add periodic transmembrane calcium exchange [Eq. (11)] into the model and determine the frequency range in which Ca^{2+} oscillations follow the external periodic forcing. The span of the frequency range in which Ca^{2+} oscillations are synchronised with the forcing signal represents the system ability to adapt the basic Ca^{2+} oscillations to the forcing signal. Therefore, the flexibility of the model system (F) is quantified by the maximal span of the frequency range in which the frequency of Ca^{2+} oscillations (ν) equals the frequency of the external forcing (ν_f):

$$F = \frac{\nu_{\max} - \nu_{\min}}{\nu_0}, \tag{14}$$

where

$$\nu_{\max} = \max\{\nu,\ \nu = \nu_f\}, \tag{15}$$

$$\nu_{\min} = \min\{\nu,\ \nu = \nu_f\}, \tag{16}$$

and ν_0 is the basic oscillation frequency of the model system without periodic forcing. By varying the forcing frequency ν_f, first $\nu_{\max}$ and $\nu_{\min}$ are determined by considering the condition $\nu = \nu_f$ in Eq. (15) and Eq. (16), respectively. Then the flexibility (F) can be simply calculated by Eq. (14). This procedure is carried out for estimating the flexibility of different chaotic and regular oscillatory regimes. High flexibility corresponds to values $F \gg 0$ whereas $F = 0$ means that the system is completely inflexible.

First, the flexibility of the chaotic regime at $r = 0.62$ is studied. The chaotic behaviour is proved by calculating the largest Lyapunov exponent, which is positive in this case (see Fig. 1). Although the regime is chaotic, a predominant frequency is well expressed as shown in Fig. 2a. The external forcing is applied to the basic Ca^{2+} oscillations. By changing the frequency of the forcing signal, we determine the range of synchronisation in which the predominant oscillation frequency of chaotic oscillations is synchronised with the forcing frequency. With high frequency forcing, we are able to enlarge the predominant frequency of the basic Ca^{2+} oscillations up to $\nu_{\max} = 1.37\ \nu_0$ (Fig. 2b). On the other hand, low forcing frequencies can reduce the basic oscillation frequency down to $\nu_{\min} = 0.72\ \nu_0$ (Fig. 2c). According to Eq. (14), this yields the flexibility of the model system $F = 0.65$. Below 72% and above 137% of the basic oscillation frequency, the synchronisation is lost and the frequency of Ca^{2+} oscillations tends to the value of the basic model system without forcing (see Fig. 3). Since the examined Ca^{2+} oscillations are of the bursting type, the synchronisation of the forcing signal with the main Ca^{2+} spike in Fig. 2b,c can also be seen as a quasi-phase-locking.

In the same way, we examine the flexibility of a regular periodic regime at $r = 0.50$. We apply the external forcing to the basic signal and determine the range of synchronisation. In contrast to the previous chaotic regime at $r = 0.62$, in this case we are only able to reduce the frequency of basic Ca^{2+} oscillations down to $\nu_{\min} = 0.97\ \nu_0$ and enlarge it up to $\nu_{\max} = 1.04\ \nu_0$ (see Fig. 3), which yields $F = 0.07$ By comparing the results obtained for the regular periodic regime at $r = 0.50$ and for the chaotic regime at $r = 0.62$, one could conclude that chaotic Ca^{2+} oscillations are more flexible than regular ones. However, the question arises if this could be generalised.

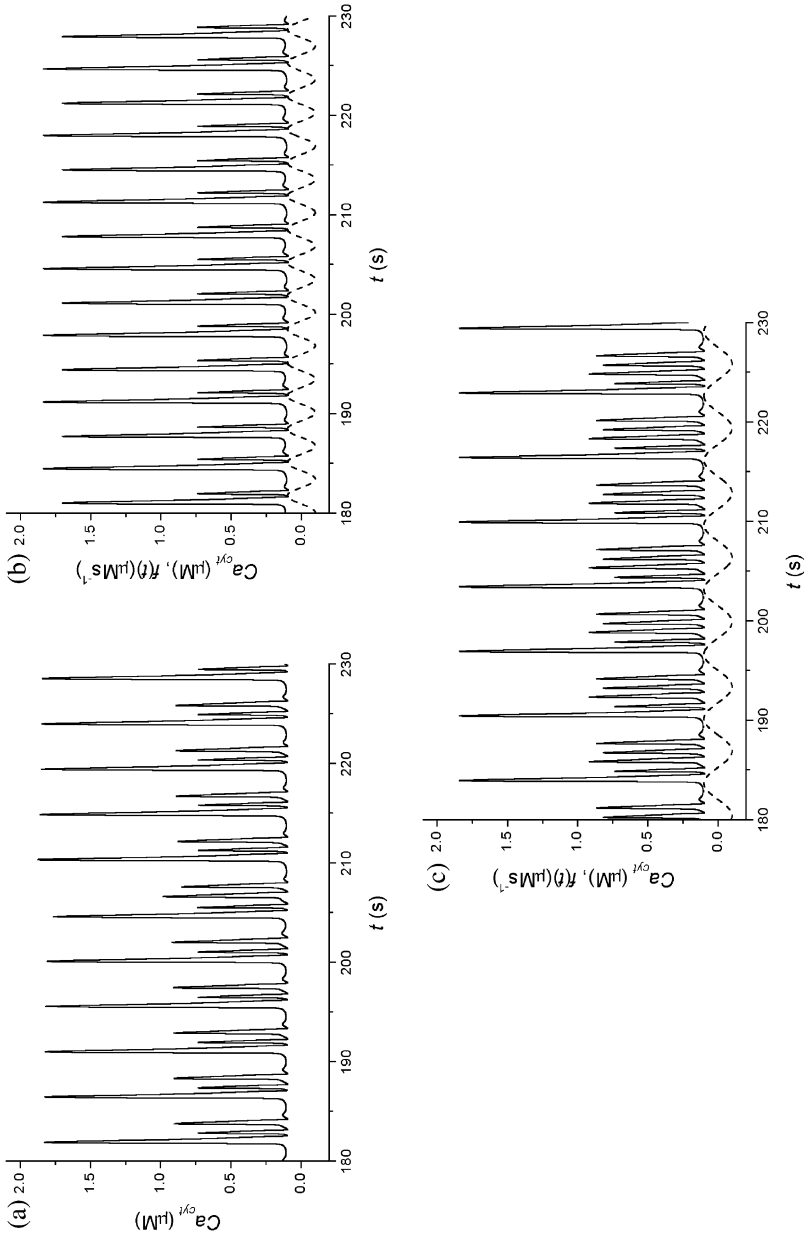

Fig. 2. Periodic forcing of chaotic Ca^{2+} oscillations at $r=0.62$. (a) Chaotic Ca^{2+} oscillations without periodic forcing. (b) Ca^{2+} oscillations (solid line) follow the forcing signal (dashed line) up to approximately 137% of their basic frequency. The amplitude of periodic forcing $f(t)$ is $a=0.1$ μM s^{-1}. (c) Ca^{2+} oscillations (solid line) follow the forcing signal (dashed line) down to approximately 72% of their basic frequency. The amplitude of periodic forcing $f(t)$ is $a=0.1$ μM s^{-1}.

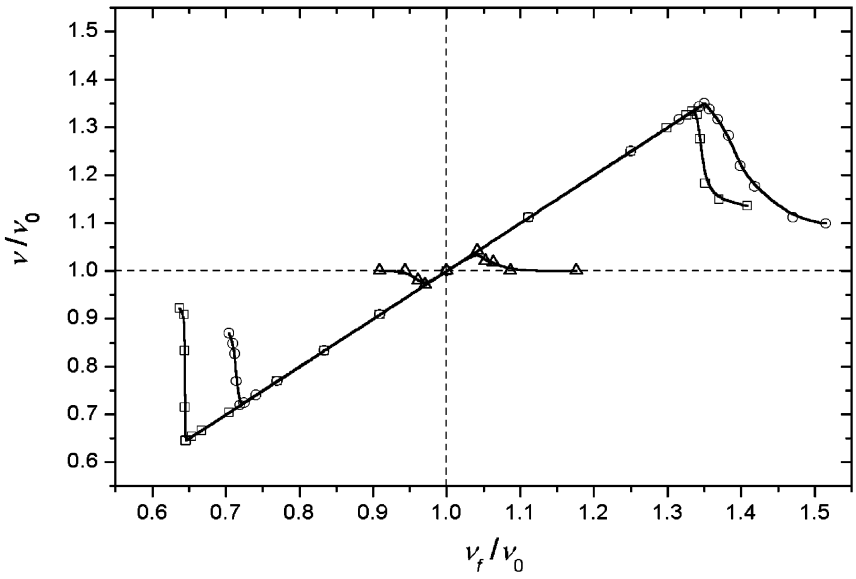

Fig. 3. Oscillation frequency ranges in which Ca^{2+} oscillations are synchronised with the forcing signal. Frequencies of forced Ca^{2+} oscillations, ν, as well as frequencies of forcing signals, ν_f, are normalised with respect to the frequency of basic Ca^{2+} oscillations, ν_0. Calculations are carried out for three different values of parameter r: (Δ) $r=0.50$, $a=0.25$ μM s^{-1}, (○) $r=0.62$, $a=0.1$ μM s^{-1} and (□) $r=0.624$, $a=0.1$ μM s^{-1}.

We investigate the flexibility of another regular Ca^{2+} oscillations at $r=0.624$. The span of the frequency range in which Ca^{2+} oscillations are synchronised with the forcing signal expands from $\upsilon_{\min}=0.64\ \nu_0$ to $\nu_{\max}=1.33\ \nu_0$ (see Fig. 3), which yields $F=0.69$. The result shows that this regular periodic regime is more flexible than the chaotic one at $r=0.62$ ($F=0.65$). In fact, the span of the frequency range in which Ca^{2+} oscillations are synchronised with the external forcing is larger for $r=0.624$ than for all other parameter values tested. It should be noted that since the amplitude of Ca^{2+} oscillations slightly decreases with increasing values of parameter r, we have accordingly reduced the amplitude of forcing from $a=0.25$ μM s^{-1} (for $r=0.50$) to $a=0.1$ μM s^{-1} (for $r=0.62$ and $r=0.624$). Herewith we avoid an amplitude effect of a fixed forcing signal.

Trying to explain the reasons for high flexibility of the model system, we analyse the attractive properties of the trajectories in phase space. The strength of the attraction is measured by the sum of Lyapunov exponents, which we calculate with the algorithm proposed by Wolf et al. [27]. The sum of Lyapunov exponents corresponds to the contraction of the phase space volume and, hence to dissipation. In Fig. 4 the dissipation of the model system is plotted vs. parameter r. By comparing Fig. 4 with the results obtained in the flexibility analysis (Fig. 3), we see that regions of weak dissipation coincide with regions of high flexibility, regardless of the complexity of Ca^{2+} oscillations. Therefore, we argue that Ca^{2+} oscillations are more flexible at weak dissipation than at high dissipation.

We tested the above-suggested criterion for flexibility of Ca^{2+} oscillations on several other model systems (not shown here). We obtained qualitatively the same results, showing that the dissipation is a useful measure for the flexibility of a dynamical system. It should be noted, however, that the sum of Lyapunov exponents gives the time-averaged dissipation of the whole attractor. Therefore, for better understanding of how the system is able to deviate from the basic attractor in phase space and herewith to adapt its oscillation frequency to the oscillation frequency of the external forcing, in addition to the time-averaged dissipation of the

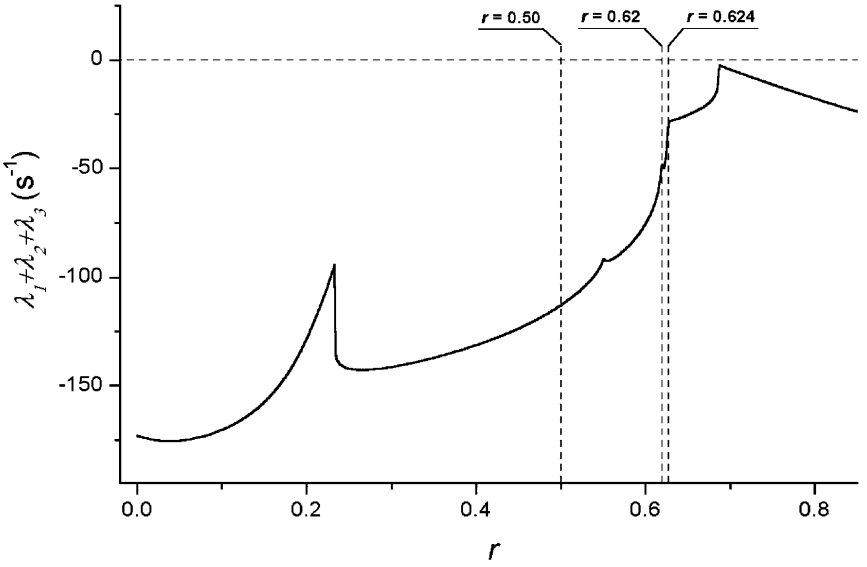

Fig. 4. Dissipation of the model system, represented as the sum of the Lyapunov exponents $(\lambda_1+\lambda_2+\lambda_3)$, is plotted vs. parameter r. Studied examples at $r=0.50$, $r=0.62$ and $r=0.624$ are marked with vertical dashed lines.

whole attractor also the time course of local dissipation along the trajectory should be considered. We demonstrate this by examples.

Fig. 5a shows the local dissipation for periodic Ca^{2+} oscillations at $r=0.68$. It can be well observed that the very low time-averaged dissipation (shown in Fig. 4) results from the sinus-like time course of the local dissipation (Fig. 5a). In accordance to our previous statement, the low time-averaged dissipation implies a high flexibility of the system, which is here expressed in a high ability of the system to respond very vividly to the external periodic forcing (see Fig. 5b). The Ca^{2+} oscillations are synchronised in a large frequency range of the forcing signal, however, the amplitude of Ca^{2+} oscillations changes considerably. For biological systems, this is not fully the desired flexibility. Biological systems must not respond just vividly but also controlled and immutably, i.e. their response has to be well defined in its frequency as well as in its amplitude. To have a flexible system with well-defined responses to the external forcing, in addition to the low time-averaged dissipation, the local dissipation has to express some asymmetry. On the larger part of the attractor, the local dissipation has to be either positive, close to zero or should oscillate around zero. This contributes to the low time-averaged dissipation and represents the flexible part of the attractor. However, in addition to this flexible part, there must also be at least one well-expressed negative dell of dissipation. This assures the system to have a strong attractive region in phase space, to which the system returns with higher probability. Herewith, the forcing signal cannot change these rigid parts of the trajectory but only alters the system behaviour in regions of low dissipation.

To demonstrate the importance of the asymmetry of local dissipation, we analyse the time course of dissipation for the highly flexible regular oscillations at $r=0.624$. Fig. 6a shows that in addition to the amplitude symmetric oscillations of the local dissipation around zero, two well-expressed negative dells of dissipation are present (marked with arrows in Fig. 6a). They act as stabilisers, which prevent uncontrolled vivid behaviour in response to the external forcing. The role of the attractive dells on one hand and the flexible, low attractive regions on the other hand can be demonstrated in

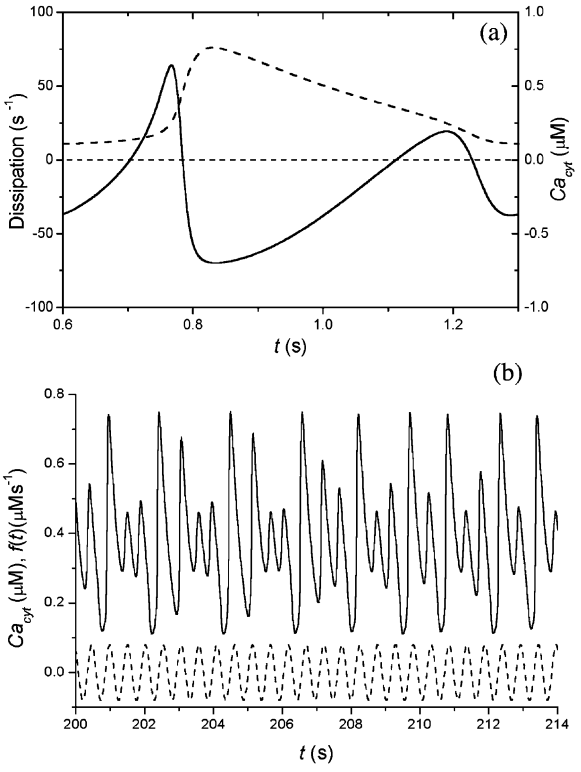

Fig. 5. Local dissipation analysis for the oscillatory regime at $r=0.68$. (a) Time course of the dissipation (solid line, left y-axis) and time course of Ca_{cyt} (dashed line, right y-axis) for one oscillation period. (b) Periodic forcing of regular Ca^{2+} oscillations at $r=0.68$. The forced Ca^{2+} oscillations (solid line) are synchronised with the forcing signal (dashed line, $\nu_f=1.35\nu_0$, $a=0.08$ μM s^{-1}); however, the amplitude of the Ca^{2+} oscillations is not well defined.

the phase space. The largest deviations of the forced trajectory from the basic attractor appear in regions of the phase space, which correspond to the most positive dissipation areas of the attractor (marked with thick lines in Fig. 6a,b). Herewith, the system is able to adjust its own frequency to the frequency of the forcing signal. However, the two well-defined negative dells (marked with arrows in Fig. 6a,b) force the system to return periodically to the strong attractive reference regions in phase space. This can be well observed in Fig. 6b where in these high attractive regions the forced trajectory coincidences with the basic attractor. Taken together, we argue that a phase space with predominantly expressed low attractive, flexible regions, and localised high attractive, rigid regions, represents a highly flexible and well-controllable system.

Our study of sensitivity and flexibility of regular and complex Ca^{2+} oscillations shows one common result, i.e. both sensitivity and flexibility do not depend on the complexity of Ca^{2+} oscillations. However, one might argue that the periodic forcing applied in studying flexibility might lead to changing from regular periodic to chaotic behaviour of

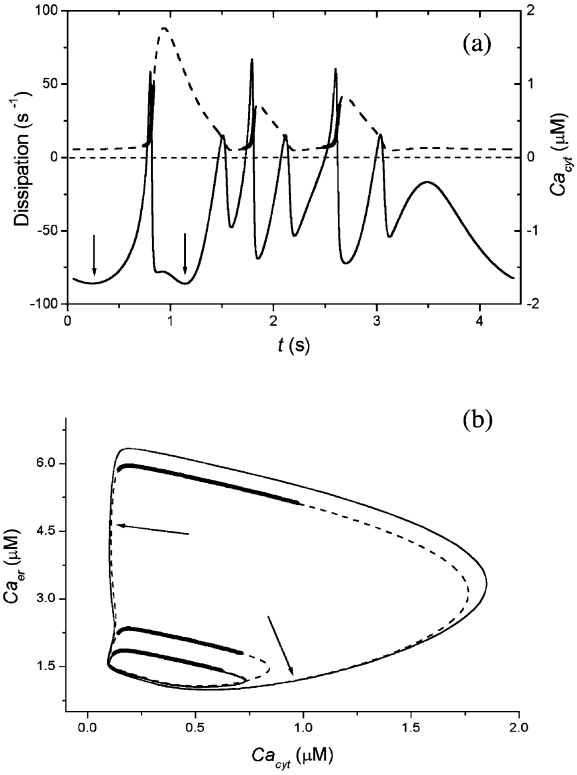

Fig. 6. Local dissipation analysis for the oscillatory regime at $r=0.624$. (a) Time course of the dissipation (solid line, left y-axis) and time course of Ca_{cyt} (dashed line, right y-axis) for one oscillation period. Thick line segments indicate regions of high flexibility. Arrows indicate two well-defined negative dells of local dissipation. (b) Limit cycles in 2D-phase space of Ca_{cyt} and Ca_{er} for $r=0.624$. The dashed line represents the limit cycle without periodic forcing (basic attractor). The thick line segments indicate regions of high flexibility whereas arrows mark the high attractive regions with low dissipation. The solid line represents the limit cycle with external periodic forcing ($\nu_f=1.25\nu_0$, $a=0.1$ μM s^{-1}).

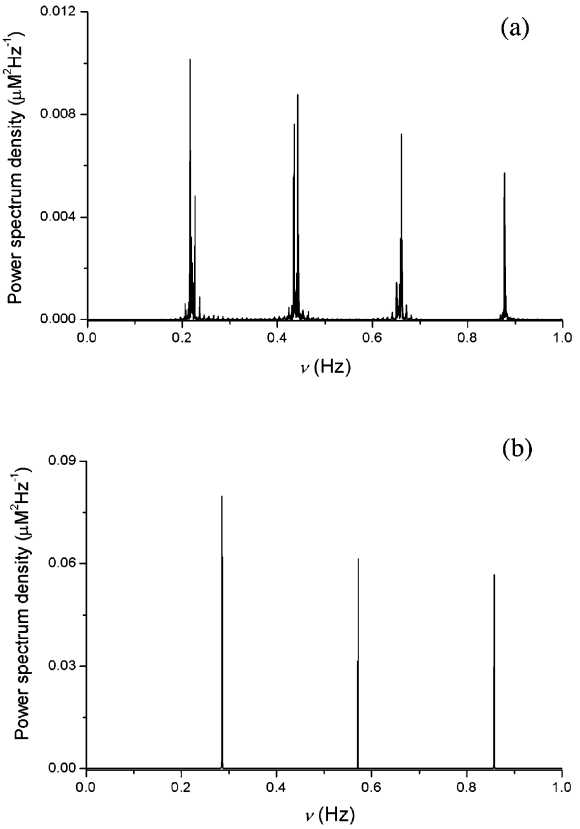

Fig. 7. Changes in power spectra caused by periodic forcing. (a) Power spectrum for chaotic Ca^{2+} oscillations at $r=0.62$. The spectrum corresponds to the time course of Ca_{cyt} presented in Fig. 2a. (b) Power spectrum for periodic forced Ca^{2+} oscillations. For parameter values see Table 1 and the caption of Fig. 2b.

the system. This would imply that chaos is nevertheless a necessary condition for high flexibility of the system. Therefore, in order to check regularity/irregularity of Ca^{2+} oscillations we have calculated power spectrums before and after applying the periodic forcing to the model system. The results show that in response to external periodic forcing the periodic regimes mostly remain regular whereas chaotic regimes most likely fall into a regular periodic regime. We demonstrate this by an example in Fig. 7. First, the power spectrum of unforced chaotic oscillations at $r=0.62$ is presented in Fig. 7a. This power spectrum corresponds to the time course in Fig. 2a. Applying the periodic forcing to these oscillations (see Fig. 2b) throws the primarily chaotic Ca^{2+} oscillations into a regular oscillatory regime. This is confirmed by the power spectrum in Fig. 7b. Similar phenomenon was also observed by Li et al. [29]. They reported about a suppression of chaotic oscillations of cyclic AMP in a suspension of Dictyostelium discoideum amoebae due to a small-amplitude periodic input of cyclic AMP.

4. Discussion

In the paper the sensitivity and flexibility of Ca^{2+} oscillations is analysed. The study was inspired by the work of Galvanovskis and Sandblom [17] arguing that chaotic Ca^{2+} oscillations seem to be more sensitive than the periodic ones. To verify this prediction we focused our study to the same mathematical model [20] and used the same basic method for analysing the sensitivity of Ca^{2+} oscillations as proposed by Galvanovskis and Sandblom [17]. However, we examined additional examples of different periodic and chaotic regimes in order to be able to make conclusions that are more general. Furthermore, we carried out an additional sensitivity test concerning changes in frequencies of Ca^{2+} oscillations. This enabled us to give further generalisations about the sensitivity of regular periodic and chaotic Ca^{2+} oscillations. To estimate the flexibility of Ca^{2+} oscillations, we proposed a new measure. We applied the external periodic forcing to the model and measured the synchronisation range in which Ca^{2+} oscillations follow the forcing signal. By this method, we analysed the flexibility of the model system comparatively for regular and irregular chaotic Ca^{2+} oscillations. The main result of our study is that the complexity of Ca^{2+} oscillations does not directly imply higher sensitivity and flexibility of Ca^{2+} oscillations in the examined mathematical model. By several examples, we showed that regular periodic regimes could be even more sensitive and flexible than chaotic ones.

The detailed analysis shows that the sensitivity of the system strongly depends on the proximity of Hopf bifurcations, regardless of the complexity of Ca^{2+} oscillations (see Table 2a,b). However, in some cases, like in the model under consideration

[20] or in the model proposed by Borghans et al. [30], for example, complex oscillations appear in the close proximity of the Hopf bifurcations. Therefore, windows of chaotic behaviour coincide with regions of high sensitivity of the system and the results obtained earlier by Galvanovskis and Sandblom [17,18] were misleading and suggested that the higher sensitivity of the system is a consequence of the chaotical behaviour.

Intuitively, the result that the proximity of a bifurcation point determines the sensitivity of the system can be well explained. By definition, bifurcations represent large qualitative changes in the system dynamics. Therefore, if the system is near a bifurcation point, small changes in parameter values can dramatically change or even stop the oscillatory behaviour. In this case, a drastic decrease in the power as well as in the oscillation frequency appears (see Table 2a,b). From the biological point of view, this is of special importance. High sensitivity of the biological system at the threshold between a stationary state and an oscillatory regime, which is linked to a considerable change in the amplitude and/or in the frequency of Ca^{2+} oscillations, plays a crucial role assuring reliable and convincing signal transduction. The considerable changes in the amplitude and/or in the frequency of Ca^{2+} oscillations depend on the type of local or global bifurcations [31,32]. Therefore, in further more detailed sensitivity analysis the type of bifurcations should also be taken into account.

In the study of how Ca^{2+} oscillations are flexible in response to external periodic forcing, we found that the flexibility of Ca^{2+} oscillations doesn't depend on their complexity. The flexibility of Ca^{2+} oscillations in the examined model system is predominantly determined by the dissipation. Our results show that Ca^{2+} oscillations are highly flexible in regimes with weak dissipation and inflexible in regimes with high dissipation. We argue that the time-averaged dissipation for the whole attractor is a suitable index in characterizing the flexibility of the system. This result can also be well interpreted intuitively. If an attractor in form of a limit cycle is weakly attractive, it seems much easier to alter its shape, thus changing the oscillation frequency of the system.

For better understanding of the system flexibility, i.e. how much the system is able to deviate from the basic attractor in phase space and herewith to adapt its frequency to the forcing frequency, in addition to the time-averaged dissipation also the time course of local dissipation along the trajectory should be considered. We showed that a flexible system with well-defined responses to the external forcing has to express some asymmetry in the local dissipation. On larger part of the attractor, the local dissipation has to be either positive, close to zero or should oscillate around zero. This contributes to the low time-averaged dissipation and represents the flexible part of the attractor. In addition to this flexible part, there must be localised but well-expressed negative dells of dissipation. They act as stabilisers and enable well-controlled responses of Ca^{2+} oscillations to the external forcing.

Attractors that are characteristic for relaxation oscillations usually express a predominantly close to zero or sinus-like time courses of dissipation with one or more well expressed negative dells of dissipation (see e.g. Fig. 6a). Intracellular calcium oscillations with their spike-like form are in general prominent examples of relaxation oscillations [20,30,33–35]. Therefore, in most cases the flexibility of Ca^{2+} oscillations can be well determined by the time-averaged dissipation only. Our results show that the analysis of the time course of dissipation is necessary for sinus-like non-relaxation type of Ca^{2+} oscillations. Although this type of Ca^{2+} oscillations is of moderate physiological importance, it appears in the majority of mathematical models. However, in the models the sinus-like Ca^{2+} oscillations are mainly a consequence of soft excitation [32,36]. Therefore, they are restricted to small parameter ranges close to special types of bifurcations and herewith have only very limited biological importance.

Our results indicate that dissipation should be considered in analysing the sensitivity and flexibility of Ca^{2+} oscillations. Also from the biological point of view, the relation between low dissipation and high flexibility of Ca^{2+} oscillations seems to be reasonable, since in view of low free energy consumption, dissipation of biological systems should be minimised [37]. However, it should

be noted that our study was made for a rather simple mathematical model [20]. Although we have additionally tested the sensitivity and flexibility criteria on some more complex mathematical models for Ca^{2+} oscillations [30,34,35] and obtained qualitatively the same results (not shown in the paper), further studies will be necessary in order to give more general conclusions. It would be interesting to find mathematical models in which the sensitivity and flexibility criteria considerably deviate from that suggested in this paper. This would give new insights into the system properties that determine high sensitivity and flexibility of dynamical systems. In further studies, it seems promising to analyse not only the attractive properties of a given limit cycle but also the attractive properties of the whole surrounding phase space. In this way, we would get a topological picture of the landscape in which the limit cycle lies, which would drastically improve our understanding of the sensitivity, flexibility and robustness of Ca^{2+} oscillations.

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