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Establishing the stochastic nature of intracellular calcium oscillations from experimental data

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Abstract

Calcium has been established as a key messenger in both intra- and intercellular signaling. Experimentally observed intracellular calcium responses to different agonists show a variety of behaviors from simple spiking to complex oscillatory regimes. Here we study typical experimental traces of calcium oscillations in hepatocytes obtained in response to phenylephrine and ATP. The traces were analyzed with methods of nonlinear time series analysis in order to determine the stochastic/deterministic nature of the intracellular calcium oscillations. Despite the fact that the oscillations appear, visually, to be deterministic yet perturbed by noise, our analyses provide strong evidence that the measured calcium traces in hepatocytes are prevalently of stochastic nature. In particular, bursting calcium oscillations are temporally correlated Gaussian series distorted by a monotonic, instantaneous, time-independent function, whilst the spiking behavior appears to have a dynamical nonlinear component whereby the overall determinism level is still low. The biological importance of this finding is discussed in relation to the mechanisms incorporated in mathematical models as well as the role of stochasticity and determinism at cellular and tissue levels which resemble typical statistical and thermodynamic effects in physics.

Keywords: Cellular signaling; Calcium oscillations; Time series analysis; Noise; Temporal correlation

1. Introduction

Many non-excitable eukaryotic cell types, including hepatocytes, respond to extracellular agonists acting through the phosphoinositide signaling pathway, such as certain hormones and neurotransmitters, by generating oscillatory changes in concentration of free cytosolic calcium (calcium oscillations). Calcium oscillations play a vital role in intra- and intercellular signaling. Many cellular processes, such as secretion or egg fertilization are regulated by oscillatory changes in cytosolic calcium concentration.

Since the 1980s, when self-sustained calcium oscillations were first discovered [1,2], numerous further experimental observations have been published (for review, see [3–5]). Calcium oscillations are generated following binding of the agonist to its plasma membrane receptor which stimulates, through G protein activation, generation of the second messenger inositol-1,4,5-trisphosphate ($InsP_3$), which activates the $InsP_3$-dependent channel on the endoplasmic reticulum leading to the rapid release of calcium ions into the cytosol. Calcium oscillations are maintained, controlled and shaped by a complex interplay of calcium fluxes between the cytosol, intracellular calcium stores, calcium-binding proteins and the external environment. Many theoretical studies have been conducted to explain the mechanism of calcium oscillations as well as the phenomenon of calcium waves. Such studies have considered influences at the level of all of the biological processes outlined above (for review see [6,7]).

The mechanisms for calcium oscillations have been mainly modeled as deterministic processes (for review see [6]). However, since the number of membrane receptors, ion channels, and calcium ions in some organelles is very low (cf. [7]), stochastic effects cannot be neglected. Indeed, it has been recognized

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recently that several different aspects of calcium signaling in cells definitely require stochastic treatment. A range of stochastic models has been developed for the modeling of single Ca^{2+} channels [7–9], intracellular calcium oscillations [10–13], and intercellular calcium wave propagation [11,14]. Some authors have also investigated stochastic effects in coupled cellular systems [11,15–18] and the role of internal noise in stochastic resonance effects [16,19–23]. To emphasize the importance of the stochastic treatment versus deterministic modeling, several comparisons of stochastic and deterministic models have been performed [12,24–28].

Although the theoretical studies predict an important role of stochasticity at the cellular level, there is a lack of direct experimental evidence confirming either the stochastic or deterministic nature of intracellular calcium signals. Therefore, in this paper the stochastic/deterministic nature of intracellular calcium oscillations is investigated directly on the basis of experimental data. We analyze experimentally measured calcium oscillations in hepatocytes by using methods of nonlinear time series analysis [29]. Previous studies have provided ample evidence that these methods can be applied successfully to experimentally obtained biomedical signals at the level of organs [30,31]. Prominent examples include the characterization of the dynamics of cardiac tissue [32], networks of neural cells [33], or the human locomotion apparatus [34,35]. Importantly, the output of these studies has vast biomedical applicability. In cardiac tissue, methods of nonlinear time series analysis can be applied to non-invasively detect "silent" heart arrhythmias or imminent heart failure, or to extract the foetal electrocardiogram from maternal recordings [36]. Moreover, electrocardiographic recordings, in conjunction with signals obtained from other cardiovascular sources (breathing, vascular rhythmicity, etc.), can be exploited to avoid mental awareness in patients during anesthesia [37,38]. In neural tissue, dynamical markers of electroencephalographic recordings can be used to diagnose epilepsy [39–41], whereas recordings obtained from the human locomotion apparatus can be used to determine neuro-degenerative diseases like Parkinson's disease, Huntington's disease, or amyotrophic lateral sclerosis [42–44]. In sum past studies analyzing experimental traces obtained at the level of organs have proved that nonlinear time series analysis methods have vast potential and applicability in various fields of medicine and biology. In this context, the present study represents a further advance in the analysis of experimental biological signals, making a step from the level of the organ towards the cellular level through the analysis of experimental recordings of intracellular calcium oscillations.

In this paper, we first present the experimental methods and measurements of cytosolic calcium concentration from single isolated hepatocytes stimulated with phenylephrine and ATP. These experimental traces are then analyzed by applying methods of nonlinear time series analysis in order to determine their stochastic/deterministic nature. We show that the analyzed intracellular calcium signals are prevalently of stochastic nature. Finally this finding is discussed in view of previously presented mathematical models and their particularities as well as analyses of oscillatory experimental traces obtained at the level of organs.

2. Experimental methods and results

Single hepatocytes were isolated from fed, male Wistar-strain rats (150-250 g) by collagenase perfusion as described previously [45]. Briefly, the hepatic portal vein was cannulated and an initial calcium-free perfusion was followed by perfusion with collagenase (0.04% w/v) and Ca^{2+} (3.8 mM) for 15 minutes. The perfusion rate was 30 ml/min throughout. The cells were harvested and incubated at 37 °C at low density (10^3 cells/ml) in 2% type IX agarose in William's medium E (WME). Single hepatocytes were prepared for microinjection with the bioluminescent calcium indicator aequorin, as described previously [46]. The injected cell was transferred to a perfusable cup held at 37 °C, positioned under a cooled, low-noise photomultiplier, and continuously superfused with WME, to which agonists were added. Photon counts were sampled every 50 ms. At the end of an experiment, the total aequorin content of each cell was determined by discharging the aequorin by lysing the cell. The data were retrospectively normalized by calculating the photon counts per second divided by the total counts remaining. The computed fractional rate of aequorin consumption could then be plotted as calcium concentration using in vitro calibration data run through a standard high frequency filter. The application of the α_1-adrenergic agonist, phenylephrine (upper panel), or ATP (lower panel) to single aequorin-injected rat hepatocytes stimulated the generation of calcium oscillations, as shown in Fig. 1.

Aequorin was provided by Prof. O. Shimomura (Marine Biological Laboratory, Woods Hole, MA, U.S.A). Collagenase

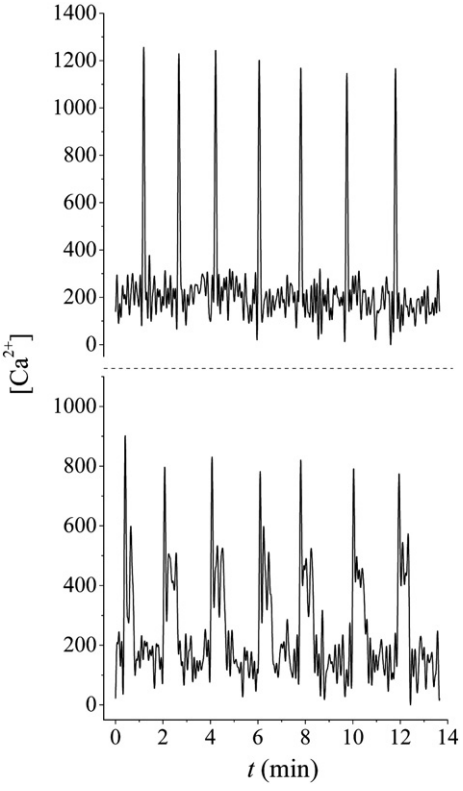

Fig. 1. Experimentally-obtained calcium responses to phenylephrine (upper trace) and ATP (lower trace) in single aequorin-injected rat hepatocytes.

was obtained from Roche Diagnostics (Lewes, U.K.) and WME from Invitrogen (Paisley, U.K.). Agarose and agonists were purchased from Sigma-Aldrich (Poole, U.K.).

3. Time series analysis methods and results

We analyze the experimental traces presented in Fig. 1 by applying methods of nonlinear time series analysis. The goal is to determine the nature of these oscillations in terms of the underlying dynamics of the system that produced it. In particular, we wish to examine whether the studied recordings of intracellular calcium oscillations are of deterministic or stochastic origin. To introduce the formalism of nonlinear time series analysis we first introduce x_i as the series under study, where x is the concentration of Ca^{2+} ions at each particular discrete time index i (note that the actual time t depicted in Fig. 1 is obtained simply by multiplying i by the sampling time interval during the experiment). First we employ surrogate data methods [47], enabling us to test different null hypotheses regarding the nature of the recordings. The three null hypotheses for which details will be presented below are: x_i are independent (temporally uncorrelated) random numbers drawn from some fixed but unknown distribution, x_i originate from a stationary linear stochastic process with Gaussian inputs, and finally, x_i originate from a stationary Gaussian linear process that has been distorted by a monotonic, instantaneous, time-independent nonlinear function. Details on surrogate data methods can be found in [29] from page 91 onwards. The main idea is that points of the original time series x_i can be altered so that some characteristic quantities of the series (like the mean, standard deviation, or the autocorrelation) are preserved while some other specific marker of nonlinearity, presently denoted by γ, changes or not. Depending on that a particular null hypothesis can be rejected or confirmed. In order to assure that the null hypothesis is not rejected solely by chance, several surrogates from the original series x_i have to be generated to achieve the desired significance level α by each test. Presently, our aim is to achieve a significance level of $\alpha=0.95$ (95%) when confirming or rejecting a null hypothesis, which means that for a single-sided test we have to generate $[1/(1-\alpha)]-1$ surrogates from x_i.

Since we wish to test whether the studied recordings of calcium oscillations are of deterministic or stochastic origin, we use as the specific marker of nonlinearity γ the zeroth-order prediction error, arguably being able to infer even very weak nonlinearities in a data set. Thus, the zeroth-order prediction error γ will be the main statistical quantity characterizing the original recordings (specifically denoted by γ_0) and the surrogates. If $\gamma_0<\gamma$ for all $[1/(1-\alpha)]-1$ generated surrogates and for all forward prediction steps n then a null hypothesis can be rejected with a significance level α. If however $\gamma_0>\gamma$ at any instance of the test the null hypothesis is said to be confirmed. The algorithm for the calculation of the zeroth-order prediction error γ in dependence on n can be found in [28] from page 44 onwards, and the actual implementation of the algorithm in C and Fortran on page 264.

As already mentioned above (and for the sake of completeness), we start with a very simple null hypothesis that the data are independent random numbers drawn from some fixed but unknown distribution. To test this null hypothesis we generate surrogates by randomly shuffling the data (without repetition), thus yielding surrogates with exactly the same distribution, yet independent construction. Finally, we calculate γ for the original recording and for every generated surrogate. Results for both studied recordings of intracellular calcium oscillations are presented in the top two panels of Fig. 2 in dependence on the number of forward prediction steps n. It is evident that γ_0 is in both cases smaller than γ pertaining to the surrogates. Moreover, this result holds for all n. We can thus clearly reject the null hypothesis that the two studied data sets are composed of independent random numbers. Note that γ pertaining to the surrogates are independent of n, which is expected, as we are dealing with independent random numbers, for which predicting one time step ahead is just as impossible as predicting thirty, a hundred or even a thousand time steps ahead. On the other hand, the increasing values of γ_0 in dependence on n suggest that there are at least some temporal correlations between data points in the two studied recordings.

We then proceed with a more interesting null hypothesis that the recordings originate from a stationary linear stochastic process with Gaussian inputs. The characteristic parameters of a time series originating from such a process are the mean, the variance, and the autocorrelation function. Thus, appropriate surrogates must consist of correlated data points with the same autocorrelation function as the original recording. In order to generate such surrogates, we have to randomize the phases of the Fourier transform of the original recording, and then perform the inverse Fourier transform to obtain the desired temporal traces. The middle two panels of Fig. 2 show γ in dependence on n. Although the trend of γ in dependence on n for the surrogates is now more closely related to the trend of γ_0, it is still obvious that γ_0 are always smaller than γ pertaining to the surrogates. Accordingly, we can also reject the null hypothesis that the two studied data sets originate from a stationary linear stochastic process with Gaussian inputs. The significance level of the rejection is the same as for the previous null hypothesis, equaling 95%.

Since the process of phase randomization preserves the Gaussian distribution, it is a common deviation from the previous null hypothesis that the data does not follow a Gaussian distribution. The most general null hypothesis, for which there is still an appropriate surrogate test developed, is that the recording originated from a stationary Gaussian linear process that has been distorted by a monotonic, instantaneous, time-independent nonlinear function. In order to generate appropriate surrogates, we employ an iterative procedure proposed by Schreiber and Schmitz [48], which uses an implementation similar to a Wiener filter to enforce the correct spectrum to the resulting surrogates. Due to this, however, a rescaling of data points was necessary to enforce the right (non-Gaussian) distribution. The two steps can be iterated several times (presently 10), whereby the resulting surrogates approach the original recording both in the spectrum as well as the distribution. As above, we have generated $[1/(1-\alpha)]-1$ such surrogates and calculated the zeroth-order prediction error γ in dependence on n. It is fascinating to discover that only spiking calcium oscillations (upper trace of Fig. 1) are able to pass the test, thus allowing the rejection of the null hypothesis. Note that in the

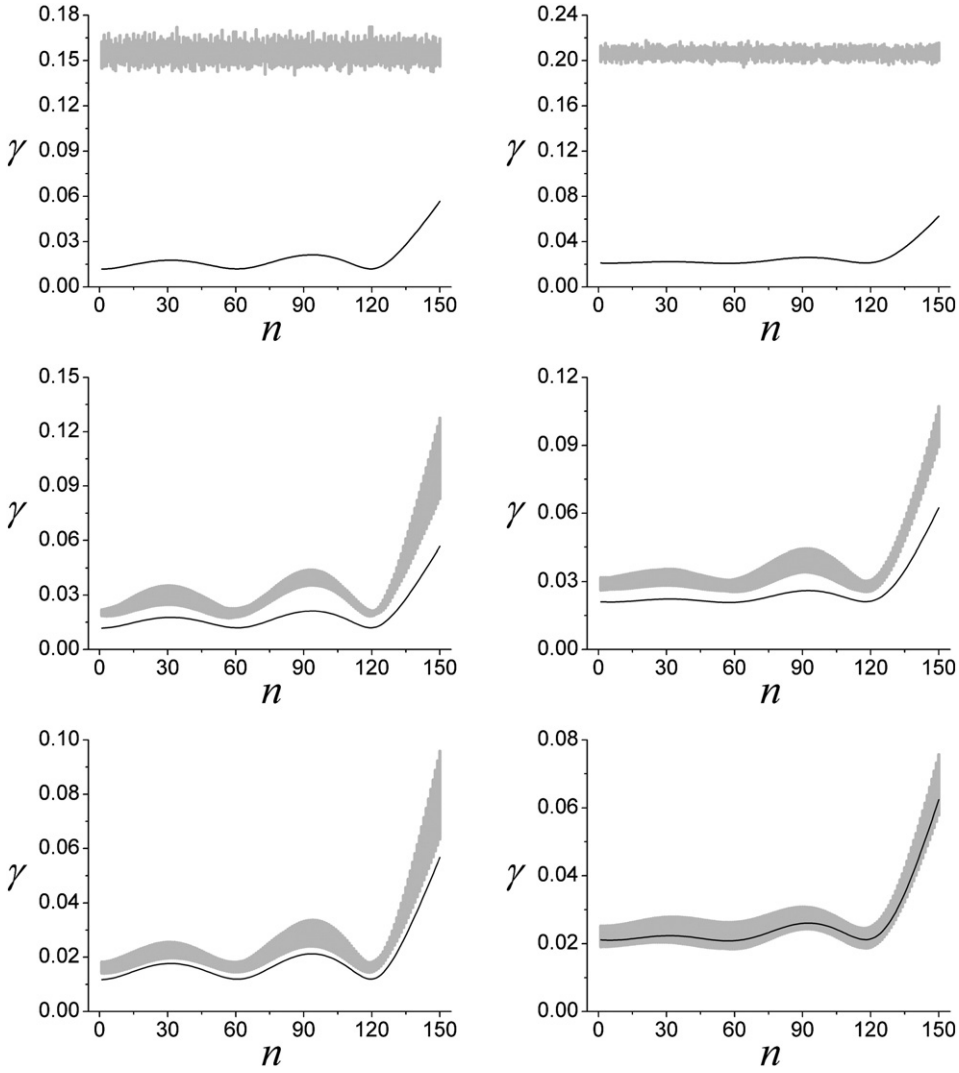

Fig. 2. Surrogate data test for different null hypotheses (see text for details). Grey stripes indicate the distribution of zeroth-order prediction errors (γ) for the surrogates, while black lines denote prediction errors for the original recordings (γ_0), in dependence on the number of prediction steps n. Results in the left panels pertain to the spiking (upper trace of Fig. 1) and in the right panels to the bursting (lower trace of Fig. 1) intracellular calcium oscillations. γ were calculated by embedding each time series into a five dimensional phase space with delay $\tau=60$ as determined by the mutual information method [50] of original recordings. Neighbors for prediction were sought amongst those points that were inside 5% of maximal distance to the reference.

bottom left panel of Fig. 2 $\gamma_0<\gamma$ irrespective of n and for all generated surrogates. On the other hand, for bursting calcium oscillations (lower trace of Fig. 1) it is in fact impossible to reject the null hypothesis (at 95% significance) that the recording originated from a stationary Gaussian linear process that has been distorted by a monotonic, instantaneous, time independent non-linear function. As can be inferred from the bottom right panel of Fig. 2, γ_0 is well within the distribution of γ.

The above results thus imply that the spiking calcium oscillations are eligible for further analyses with methods of nonlinear time series analysis. It is thus reasonable to proceed with a standard determinism test to determine the level of stochasticity in the upper trace of Fig. 1. We used the method originally proposed by Kaplan and Glass [49], which is based on measuring average directional vectors in a coarse-grained phase space. The idea is that, in case of a deterministic solution, neighboring trajectories in a small portion of the phase space should all point in the same direction, *i.e.* not cross, thus assuring uniqueness of solutions, which is the hallmark of determinism. The determinism factor $0\leq\kappa\leq1$ is obtained by calculating the average length of all resultant vectors pertaining to a particular phase space box, whereby the resultant vectors are obtained by assigning a unit vector to each pass of the trajectory through a particular phase space box and calculating their vector sum. Hence, if the dynamics of oscillations is deterministic, the average length of all directional vectors κ will be 1, while for a completely stochastic system $\kappa=0$. In order to employ the method we reconstruct the phase space from x_i via the standard embedding procedure [29]

$$\mathbf{p}_i = \left(x_i, x_{i+\tau}, x_{i+2\tau}, \ldots, x_{i+(m-1)\tau}\right), \tag{1}$$

using the embedding delay $t=60$ [50] and embedding dimension $m=7$ [51] (we have used $m=5$ for results in Fig. 2

to relax the suitable neighbors criterion for the surrogates, but results remain qualitatively the same if higher embedding dimensions are used). The phase space $\mathbf{p}_i$ was coarse-grained into 16^7 boxes and the pertaining determinism factor was found equaling $\kappa \approx 0.7$, thus indicating that although the temporal trace might have been produced by dynamic nonlinearities, the level of stochasticity is still high as experimental recordings of deterministic signals at the organ level usually have $\kappa > 0.9$ [52,53].

4. Discussion

In summary, the results of our analyses suggest that stochasticity is an important factor in the dynamics of intracellular calcium oscillations. Particularly, the presented results of nonlinear time series analysis methods applied to experimental calcium traces in hepatocytes show an extremely high degree of stochasticity at the cellular level. Although surprising at the first glance, these results represent an experimentally based confirmation of some previous theoretical hypotheses. There exist numerous theoretical predictions drawing attention to stochastic modeling and emphasizing the importance of stochastic effects at the cellular level [7–23]. It has been shown that dynamics of single Ca^{2+} channels can be better described by stochastic modeling [7–9]. Stochastic effects were also included in mathematical models for intercellular calcium oscillations [10–13], intracellular calcium wave propagation [11,14], and coupled cellular systems [11,15–18]. Moreover, it has also been shown that inherent fluctuations or noisy environment might lead to stochastic resonance effects [16,19–23]. It should be emphasized that some of these model predictions are pure theoretical considerations; however, as our results show, there exist, at least for stochastic modeling of calcium oscillations, a solid experimental base. This again confirms the strong predictive power of mathematical modeling. Several examples exist in which mathematical models had indicated solutions, which were confirmed experimentally later [54]. Therefore, interactive experimental and theoretical investigations are of crucial importance. Indeed, the fairly small number of Ca^{2+} ions within the cell (cf. [7]) creates associated non-negligible stochastic fluctuations. Together with the extensive measurement error inevitably present in such complex experimental set-ups, this will contribute substantially to temporal traces that show very sparse markers of dynamic nonlinearity.

Previously, nonlinear time series analysis of biological signals has been limited to those measured at the level of the organ. Examples range from the analyses of electrocardiographic recordings [32,55] as well as other cardiovascular sources such as breathing and vascular rhythmicity [38], electroencephalographic recordings [33], or recordings characterizing the human locomotion apparatus [34,35]. These studies show that signals measured at the level of the organ are characterized by a high degree of determinism, much higher than that characterizing the cellular signals presented here. This apparent discrepancy between the stochastic nature of cellular signals and deterministic nature of signals in tissue compares well with the scenario recently predicted by mathematical modeling of coupled calcium oscillators [17]. In that theoretical study a mechanism is presented for the transition from stochasticity to determinism in calcium oscillations, via diffusive coupling of individual cells that are modeled by stochastic simulations of the governing reaction-diffusion equations. It has been shown that, under physiologically-relevant conditions, the collective dynamics of coupled cells is, unlike that of isolated cells, deterministic for large-enough ensemble sizes. These model predictions are in best agreement with the nonlinear time series analysis of experimental results at the cellular level and at the level of the organ, i.e., stochastic versus deterministic nature between real-life recordings of physiological functions at cellular and organ level.

The stochastic nature of signals at the cellular level and the deterministic nature of signals at the level of the organ also compares well with the relation of statistical physics and thermodynamics. It is well known that predictions obtained by statistical methods approach the deterministic limit for large particle numbers. By using this analogy, it can be hypothesized that the stochastic nature of signals at cellular level becomes increasingly deterministic when the ensemble of cells forming the tissue increases. To test the hypothesis, further experimental measurements accompanied by nonlinear time series analysis are needed; in particular recordings from different numbers of cells. It would then be interesting to analyze the transition from single-cell traces to recordings at the level of the organ, and thus to elucidate directly from the experimental data, the discrepancy between stochasticity at the cellular level, and the prevalence of determinism at the level of the organ.

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References

[1] K.S.R. Cuthbertson, P.H. Cobbold, Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca^{2+}, Nature 316 (1985) 541–542.
[2] N.M. Woods, K.S.R. Cuthbertson, P.H. Cobbold, Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes, Nature 319 (1986) 600–602.
[3] A. Goldbeter, Biochemical Oscillations and Cellular Rhythms, Cambridge University Press, Cambridge, 1996.
[4] M. Berridge, P. Lipp, M. Bootman, Calcium signaling, Curr. Biol. 9 (1999) R157–R159.
[5] A.K. Green, O. Zolle, A.W.M. Simpson, Regulation of $[Ca^{2+}]_c$ oscillations by plasma membrane Ca^{2+} fluxes: a role for natriuretic peptides, Biochem. Soc. Trans. 31 (2003) 934–938.
[6] S. Schuster, M. Marhl, T. Höfer, Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signaling, Eur. J. Biochem. 269 (2002) 1333–1355.
[7] M. Falcke, Reading the patterns in living cells — the physics of Ca^{2+} signaling, Adv. Phys. 53 (2004) 255–440.
[8] J.W. Shuai, P. Jung, Optimal intracellular calcium signaling, Phys. Rev. Lett. 88 (2002) 068102.
[9] F.M. Gabhann, M.T. Yang, A.S. Popel, Monte Carlo simulations of VEGF binding to cell surface receptors in vitro, Biochim. Biophys. Acta 1746 (2005) 95–107.

[10] M. Kraus, B. Wolf, Modellbildung in der Biologie: Strukturierte Analyse intrazellulärer Calcium-Oszillationen in elektrisch nicht erregbaren Zellen, Naturwissenschaften 79 (1992) 289–299.
[11] M.E. Gracheva, R. Toral, J.D. Gunton, Stochastic effects in intercellular calcium spiking in hepatocytes, J. Theor. Biol. 212 (2001) 111–125.
[12] U. Kummer, B. Krajnc, J. Pahle, A.K. Green, C.J. Dixon, M. Marhl, Transition from stochastic to deterministic behavior in calcium oscillations, Biophys. J. 89 (2005) 1603–1611.
[13] K. Prank, M. Waring, U. Ahlvers, A. Bader, E. Penner, M. Möller, G. Brabant, C. Schöfl, Precision of intracellular calcium spike timing in primary rat hepatocytes, Syst. Biol. 2 (2005) 31–34.
[14] M. Kraus, B. Wolf, Crosstalk between cellular morphology and calcium oscillation patterns, Cell Calcium 19 (1996) 461–472.
[15] M.E. Gracheva, J.D. Gunton, Intercellular communication via intracellular calcium oscillations, J. Theor. Biol. 221 (2003) 513–518.
[16] J. Zhang, Z. Hou, H. Xin, Effects of internal noise for calcium signalling in a coupled cell system, Phys. Chem. 7 (2005) 2225–2228.
[17] M. Perc, M. Gosak, M. Marhl, From stochasticity to determinism in the collective dynamics of diffusively coupled cells, Chem. Phys. Lett. 421 (2006) 106–110.
[18] M. Perc, M. Marhl, Noise-induced spatial dynamics in the presence of memory loss, Physica, A 375 (2007) 72–80.
[19] R. Steuer, C. Zhou, J. Kurths, Constructive effects of fluctuations in genetic and biochemical regulatory systems, Biosystems 72 (2003) 241–251.
[20] H. Li, Z. Hou, H. Xin, Internal noise stochastic resonance for intracellular calcium oscillations in a cell system, Phys. Rev. E 71 (2005) 061916.
[21] H. Li, Z. Hou, H. Xin, Internal noise enhanced detection of hormonal signal through intracellular calcium oscillations, Chem. Phys. Lett. 402 (2005) 444–449.
[22] M. Yi, Q. Liu, J. Li, C. Zhu, Enhancement of internal-noise coherence resonance by modulation of external noise in a circadian oscillator, Phys. Rev. E 73 (2006) 041923.
[23] C. Zhu, Y. Jia, Q. Liu, L. Yang, X. Zhan, A mesoscopic stochastic mechanism of cytosolic calcium oscillations, Biophys. Chem. 125 (2007) 201–212.
[24] D. Gonze, J. Halloy, A. Goldbeter, Deterministic versus stochastic models for circadian rhythms, J. Biol. Phys. 28 (2002) 637–653.
[25] D. Gonze, J. Halloy, J.C. Leloup, A. Goldbeter, Stochastic models for circadian rhythms: effect of molecular noise on periodic and chaotic behaviour, C. R., Biol. 326 (2003) 189–203.
[26] D. Gonze, J. Halloy, A. Goldbeter, Emergence of coherent oscillations of stochastic models for circadian rhythms, Physica A 342 (2004) 221–233.
[27] P. Smolen, D.A. Baxter, J.H. Byrne, A reduced model clarifies the role of feedback loops and time delays in the *Drosophila* circadian oscillator, Biophys. J. 83 (2002) 2349–2359.
[28] D.B. Forger, C.S. Peskin, Stochastic simulation of the mammalian circadian clock, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 321–324.
[29] H. Kantz, T. Schreiber, Nonlinear Time Series Analysis, Cambridge University Press, Cambridge, 1997.
[30] A.L. Goldberger, L.A.N. Amaral, L. Glass, J.M. Hausdorff, P.C. Ivanov, R.G. Mark, J.E. Mietus, G.B. Moody, C.-K. Peng, H.E. Stanley, PhysioBank, PhysioToolkit, and PhysioNet: components of a new research resource for complex physiologic signals, Circulation 101 (2000) e215–e220.
[31] A.L. Goldberger, L.A.N. Amaral, J.M. Hausdorff, P.C. Ivanov, C.K. Peng, H.E. Stanley, Fractal dynamics in physiology: alterations with disease and aging, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 2466–2472.
[32] H. Kantz, T. Schreiber, The human ECG — nonlinear deterministic versus stochastic aspects, IEE Proc., Sci. Meas. Technol. 145 (2002) 279–284.
[33] A. Galka, Topics in Nonlinear Time Series Analysis: With Implications for EEG Analysi, World Scientific Publishing Company, Singapore, 2000.
[34] J.B. Dingwell, J.P. Cusumano, Nonlinear time series analysis of normal and pathological human walking, Chaos 10 (2000) 848–863.
[35] U.H. Buzzi, N. Stergiou, M.J. Kurz, P.A. Hageman, J. Heidel, Nonlinear dynamics indicates aging affects variability during gait, Clin. Biomech. 18 (2003) 435–443.
[36] M. Richter, T. Schreiber, D.T. Kaplan, Fetal ECG extraction with nonlinear state-space projections, IEEE Trans. Biomed. Eng. 45 (1998) 133–137.
[37] G. Widman, T. Schreiber, B. Rehberg, A. Hoeft, C.E. Elger, Quantification of depth of anesthesia by nonlinear time series analysis of brain electrical activity, Phys. Rev. E 62 (2000) 4898–4903.
[38] A. Stefanovska, H. Haken, P.V.E. McClintock, M. Hožič, F. Bajrović, S. Ribarič, Reversible transitions between synchronization states of the cardiorespiratory system, Phys. Rev. Lett. 85 (2000) 4831–4834.
[39] J. Theiler, On the evidence for low-dimensional chaos in an epileptic electroencephalogram, Phys. Lett. A 196 (1994) 335–341.
[40] M.W. Slutzky, P. Cvitanovic, D.J. Mogul, Deterministic chaos and noise in three in vitro hippocampal models of epilepsy, Ann. Biomed. Eng. 29 (2001) 607–618.
[41] Z.J. Kowalik, A. Schnitzler, H. Freund, O.W. Witte, Local Lyapunov exponents detect epileptic zones in spike-less interictal MEG recordings, Clin. Neurophysiol. 112 (2001) 60–67.
[42] J.M. Hausdorff, S.L. Mitchell, R. Firtion, C.K. Peng, M.E. Cudkowicz, J.Y. Wei, A.L. Goldberger, Altered fractal dynamics of gait: reduced stride-interval correlations with aging and Huntington's disease, J. Appl. Physiol. 82 (1997) 262–269.
[43] J.M. Hausdorff, A. Lertratanakul, M.E. Cudkowicz, A.L. Peterson, D. Kaliton, A.L. Goldberger, Dynamic markers of altered gait rhythm in amyotrophic lateral sclerosis, J. Appl. Physiol. 88 (2000) 2045–2053.
[44] R. Yulmetyev, S. Demina, N. Emelyanova, F. Gafarova, P. Hänggi, Stratification of the phase clouds and statistical effects of the non-Markovity in chaotic time series of human gait for healthy people and Parkinson patients, Physica, A 319 (2003) 432–446.
[45] C.J. Dixon, P.H. Cobbold, A.K. Green, Actions of ADP, but not ATP, on cytosolic free Ca^{2+} in single rat hepatocytes mimicked by 2-methylthioATP, Br. J. Pharmacol. 116 (1995) 1979–1984.
[46] C.J. Dixon, A.K. Green, Measuring calcium and calmodulin inside and outside cells, in: O.H. Petersen (Ed.), Aequorin measurements of cytosolic calcium, Springer Lab Manual, Springer-Verlag, New York, 2001, pp. 65–90.
[47] J. Theiler, S. Eubank, A. Longtin, B. Galdrikian, J.D. Farmer, Testing for nonlinearity in time series: the method of surrogate data, Physica, D 58 (1992) 77–94.
[48] T. Schreiber, A. Schmitz, Improved surrogate data for nonlinearity tests, Phys. Rev. Lett. 77 (1996) 635–638.
[49] D.T. Kaplan, L. Glass, Direct test for determinism in a time series, Phys. Rev. Lett. 68 (1992) 427–430.
[50] A.M. Fraser, H.L. Swinney, Independent coordinates for strange attractors from mutual information, Phys. Rev., A 33 (1986) 1134–1140.
[51] M.B. Kennel, R. Brown, H.D.I. Abarbanel, Determining embedding dimension for phase space reconstruction using a geometrical construction, Phys. Rev., A 45 (1992) 3403–3411.
[52] M. Perc, Nonlinear time series analysis of the human electrocardiogram, Eur. J. Phys. 26 (2005) 757–768.
[53] M. Perc, The dynamics of human gait, Eur. J. Phys. 26 (2005) 525–534.
[54] S. Schuster, E. Klipp, M. Marhl, Discovering biomolecular mechanisms with computational biology, in: F. Eisenhaber (Ed.), The predictive power of molecular network modelling case studies of predictions with subsequent experimental verification, Lands Bioscience, Georgetown, 2006, pp. 115–127.
[55] T. Jagrič, M. Marhl, D. Štajer, Š. Tadel Kocjančič, T. Jagrič, M. Podbregar, M. Perc, Irregularity test for very short ECG signals as a method for predicting a successful defibrillation in patients with ventricular fibrillation, Transl. Res. 149 (2007) 145–151.