

Stochastic noise interferes coherently with a model biological clock and produces specific dynamic behaviour

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The influence of noise is unavoidable in all living systems. Its impact on a model of a biological clock, normally running in regular oscillating modes, is examined. It is shown that in a specific system in which endogenous rhythmicity is produced by a beat oscillator acting on a feedback coupled metabolic pool system, noise can act coherently to produce unexpected dynamic behaviour, running from regular over pseudo-regular to irregular time-structures. If the biological system consists of a set of identical weakly coupled cells, stochasticity may lead to phase decoupling producing irregular spatio-temporal patterns. Synchronization via phase resetting can be achieved by external short-time temperature pulses. Explicit results are obtained for the well-studied circadian photosynthesis oscillations in plants performing crassulacean acid metabolism. Because of the generic structure of the underlying nonlinear dynamics they can, however, be regarded as a general property of the influence of noise on nonlinear excitable systems with fixed points occurring close to limit cycles.

Keywords: stochastic noise; biological clock; crassulacean acid metabolism; endogenous rhythm; limit cycle oscillation; phase resetting

1. INTRODUCTION

Evolution created biological rhythms as important elements for the adjustment of living species to regularly changing environmental conditions or internal synchronization. In most cases, they are characterized by free-running, endogenous rhythms, ranging from periods of seconds (e.g. heartbeat) to years (e.g. population dynamics). They can adjust themselves in a certain entrainment range to varying external influences, which thereby gives them greater plasticity for adaptation to changes in the environment than a rigid coupling could accomplish. The physiological realization very often consists of metabolic pools with biochemical reaction chains, nonlinearly coupled and regulated by feedback loops. The period of the free-running oscillation is either determined by the kinetic constants which govern the biochemical reactions, or by a hysteresis switch (beat oscillator) which can be realized, e.g. in membrane transport processes (Lüttge & Beck 1992).

At finite temperature, noisiness is an unavoidable by-product in dynamic systems. Normally, noise is regarded as a disturbance, leading to unpredictable irregularities in oscillating systems. This has led people over many years to attribute irregular time-structures to stochasticity, produced either by the measurement or by the system itself, thus containing no more deterministic elements worthy of investigation, and being a nuisance better discarded. Recent studies in nonlinear dynamics, however, have shown that there can be constructive interference between regular dynamics and noise, leading to

processes called stochastic resonance and stochastic coherence (Wiesenfeld & Moss 1995, Pikovsky & Kurths 1997). The question therefore arises of whether such cooperative effects could play a role in the behaviour of biological clocks and which consequences have to be expected in their response to external changes in the control parameters.

In this work we investigate the influence of stochastic noise on endogenous rhythmicity in the special case of photosynthesis oscillations in plants performing crassulacean acid metabolism (CAM), where CO₂ is prefixed by phosphoenolpyruvate carboxylase (PEPc) and, independent of light, stored in the form of malate (or malic acid) in the cell vacuole. Subsequently, it is remobilized and decarboxylated internally, producing CO₂ for reduction and assimilation in the Calvin cycle (Winter & Smith 1996). Extensive experimental data and reliable theoretical models exist for the CAM plant *Kalanchoë daigremontiana* Hamet et Perrier de la Bâthie (Lüttge & Beck 1992, Grams *et al.* 1996, Blasius *et al.* 1997). This ensures that our results stay as close as possible to reality, although, by the general nature of the underlying dynamics, they can nevertheless serve as a generic example for the influence of noise on nonlinear biological oscillators. Section 2 introduces our minimal model, derived earlier as a consequence of largely different metabolic reaction times, which emphasizes the role of the tonoplast membrane as the principal period-determining step for endogenous oscillations. The phase space structure of the model, showing the occurrence of fixed points and limit cycles in their dependence on external control parameters, is outlined in Blasius *et al.* (1998, 1999). In §3 we add stochastic noise to the dynamics, discuss their consequences for the rhythmic structure and compare them with experimental results, while §4 contains our conclusions.

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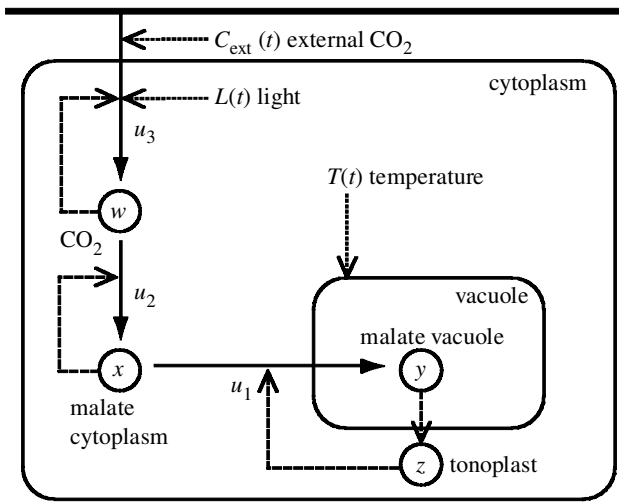


Figure 1. Block diagram of the CAM model showing as dynamic variables the three reactant pools, w , x , y , and a variable characterizing the order of the lipid molecules in the tonoplast (Neff *et al.* 1998), z (circled), within a CAM cell. They are connected by flows u_1 , u_2 , u_3 (solid arrows). Regulatory feedback loops are indicated as dashed lines. The model depends on three external control parameters: temperature, T , light intensity, L , and external CO₂ concentration, C_{ext} (dotted arrows).

2. THE MINIMAL CAM MODEL

The CAM model which we will use has been described extensively in earlier work (Blasius *et al.* 1997, 1998, 1999), and here we only outline the connections briefly. The model can be characterized by the block diagram presented in figure 1. It shows the major reactant pools of CAM which generate the carbon flow during the circadian cycle. The pool concentrations are the following: internal CO₂ concentration, w ; malate concentration in the cytoplasm, x ; malate concentration in the vacuole, y ; while z is a variable which describes the ordering of the lipid molecules in the tonoplast membrane (Neff *et al.* 1998). These are the dynamic variables of the cyclic process. They are connected by the flows, u_1 , u_2 , u_3 , defining the gain and loss terms of the metabolites.

In our model, regulation of the transport of malate between the cytoplasm and vacuole is the key process for establishing the observed endogenous rhythm in CAM, and it requires a hysteretic switching of the passive malate efflux. We have studied the behaviour of the tonoplast, which separates the vacuole from the cytoplasm, as a lipid membrane under the influence of temperature and osmotic stress during the filling process of the vacuole by active influx of malate. We could establish a thermodynamic phase diagram relating the order parameter, z , of the membrane to temperature and the surface density of lipid molecules (Neff *et al.* 1998). Incorporating this into the CAM model introduces z as a new dynamic variable, leading to an additional equation for its time change (Blasius *et al.* 1999).

The dynamics are characterized by a set of four coupled, nonlinear differential equations of first order in time:

$$\begin{aligned}\varepsilon \dot{w} &= -u_2 + u_3, \\ \varepsilon \dot{x} &= -u_1 + u_2, \\ \dot{y} &= +u_1, \\ \tau \dot{z} &= g(z, T) - y.\end{aligned}\quad (1)$$

The fourth equation governs the dynamics of the tonoplast order, where the function $g(z, T)$ is obtained from the membrane model (Neff *et al.* 1998), and τ is the time-constant for relaxation into thermal equilibrium (Blasius *et al.* 1999). The smallness parameter $\varepsilon \ll 1$ reflects the volume ratio of cytoplasm to vacuole, which is typically of the order of 1/100 in CAM plants. It is decisive for the time-structure of CAM which resembles the form of a relaxation oscillator (Murray 1993), and it has the consequence that the dynamics of the reactant pools in the cytoplasm, w , x , are held permanently in a pseudo steady state (Blasius *et al.* 1998).

The flows u_i involve modelling of the metabolic reactions and thus comprise the whole structure of the carbon circulation in CAM. According to our previous work, the biochemical processes are well described by the following flow structure:¹

$$\begin{aligned}u_1 &= c x - \frac{y}{z} \\ u_2 &= \frac{w}{x} - x \\ u_3 &= c_J \frac{(C_{\text{ext}}(t) - w)}{\exp(\alpha w)} - L(t) w + c_R \frac{L_K}{L(t) + L_K} \cdot \frac{w_\Gamma}{w + w_\Gamma}.\end{aligned}\quad (2)$$

Here, u_1 is the difference between malate influx and efflux into and out of the vacuole, modelled with the dynamic hysteresis switch (Blasius *et al.* 1999), and u_2 describes the difference between malate production from CO₂ fixation by PEPc and its depletion by decarboxylation. The well-known and important feedback inhibition of PEPc by malate is taken care of in the $1/x$ -dependence of the first term. It leads to rhythmic changes in the activity of PEPc, and is essential for CAM to activate the Calvin cycle (Nimmo *et al.* 1987). Finally, u_3 models CO₂ uptake from outside, $C_{\text{ext}}(t)$, minus CO₂ consumption by photosynthesis, which is directly proportional to the external control parameter light intensity, $L(t)$, plus CO₂ production by respiration. Regulation of CO₂ uptake by stomatal opening and closing is due to a stomatal resistance with inverse exponential dependence on internal CO₂ concentration, growth rate α , and multiplying the CO₂ uptake. An additional CO₂ source from respiration is provided by the last term in the equation for u_3 . Respiration is assumed to be inhibited by light and internal CO₂, with inhibition constants L_K and w_Γ , respectively. In addition to these regulation elements between pools, indicated as dashed lines in figure 1, flows depend linearly on substrate concentrations, with material constants which are reduced to three (c , c_J , c_R) in the nondimensional formulation. Consequently, we also present numerical results in nondimensional units.

The fixed points of the reactant pools can be obtained by setting each flow in equation (1) to zero individually,

$$u_i = 0, \quad (i = 1 \dots 3), \quad (3)$$

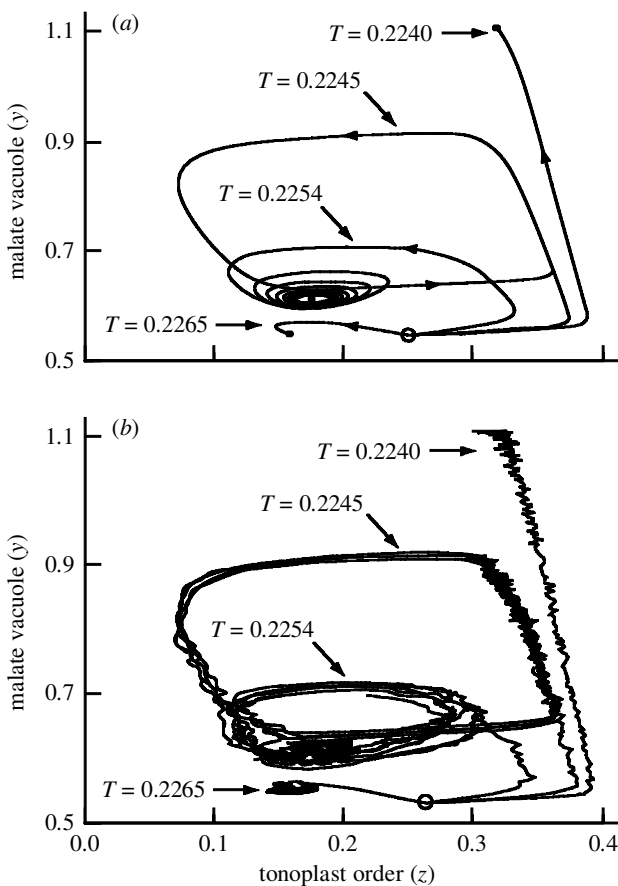


Figure 2. Trajectories in the (z, y) -plane for different relative temperatures and all starting at the same point. Four types are shown. For the low temperature $T = 0.2240$ the trajectory runs into the upper fixed point, characterized by full vacuole $y \approx 1.1$. The trajectory reaches the lower fixed point $y \approx 0.6$ with a nearly empty vacuole for the high temperature $T = 0.2254$ on a spiral path. For even higher temperature $T = 0.2265$ the fixed point at $y \approx 0.55$ is reached on a direct path. At intermediate temperatures, here exemplified by $T = 0.2245$, limit cycle oscillations occur. (a) Trajectories without noise (open circle, starting point; black dots, fixed points; arrows show direction of motion along trajectories). (b) The same trajectories adding a noise component as in equation (4). The trajectories in the vicinity of the low and the very high temperature fixed points are little affected. The trajectory for $T = 0.2254$, without noise running into the fixed point lying close to the limit cycle regime is, however, strongly disturbed. Noise kicks throw it stochastically into nearby limit cycles from where it returns to the fixed point only after a full cycle. (Control parameters: $C_{\text{ext}} = 1$, $L(t) = 1$; constants: $c = 5.5$, $c_f = 1$, $c_R = 1$, $\varepsilon = 0.001$, $\alpha = 1.5$, $w_\Gamma = 0.1$, $L_K = 0.5$, noise strength: $R = 0.1$; parameters in $g(z, T)$ (Neff *et al.* 1998): $\phi_0 = 2$, $\lambda = 7$).

whereas the equilibrium of the membrane order is defined by the condition $\dot{z} = 0$. As has been discussed by Blasius *et al.* (1999), these conditions define the stable fixed points and the limit cycles of the CAM dynamics in our model. For rather high and low temperatures, stable fixed points, defining steady states of the system, emerge, while for medium temperatures, oscillating solutions exist in the form of limit cycles (Murray 1993). This reflects exactly the experimental situation that the CAM rhythm disappears in both the high and in the low temperature regimes (Grams *et al.* 1997).

Figure 2a shows the phase space trajectories of the CAM cycle in continuous light with temperature as the parameter, plotted in the (z, y) -plane. For low temperatures, the trajectory evolves towards stable fixed points characterized by a high malate content of the vacuole ('full' vacuole). Conversely, at the upper end of the temperature range the rhythm stops with an 'empty' vacuole. Limit cycle oscillations are found in the medium temperature range only. The close proximity of fixed points and limit cycles (figure 2a) suggests that the influence of noisy fluctuations may lead to dramatic effects on the time-structure of CAM.

3. INFLUENCE OF STOCHASTIC NOISE

In all macroscopic systems the influence of noise is unavoidable. At low temperature quantum noise prevails, while at higher temperature thermal noise is present which may be enhanced in nearly unstable systems by spontaneous kicks into the unstable region. Normally, one tries to minimize the influence of noise by measures which increase the signal-to-noise (S/N) ratio. Only recently it has been realized that in nonlinear systems noise by itself can act constructively on external or internal signals and, depending on the noise level, enhance the S/N ratio, i.e. elicit stochastic resonance and stochastic coherence, respectively (Wiesenfeld & Moss 1995, Pikovsky & Kurths 1997).

To investigate the influence of noise on our CAM model, we add a noise term $\eta(t)$ to the fourth of equations (1). This leads to the Langevin equation (Van Kampen 1981)

$$\tau \dot{z} = g(z, T) - y + \eta(t). \quad (4)$$

For the following numeric results we use Gaussian delta-correlated noise with zero mean and intensity R :

$$\langle \eta(t) \rangle = 0, \quad \langle \eta(t) \eta(t') \rangle = R \delta(t - t'). \quad (5)$$

Figure 2b depicts the simulation results in continuous light in the (y, z) -plane for different temperatures. Compared with the dynamics without noise (figure 2a), there is not much change for most temperatures. Noise leads to a quivering of the trajectories around the zero noise curve. Only in the small temperature regime corresponding to the transition between limit cycle oscillations and a steady state does the system respond in a sensitive way to the addition of noise. If, under the influence of noise, the trajectory in this temperature regime is perturbed beyond a certain critical threshold distance from the fixed point, it cannot spiral back directly into the equilibrium but has to undergo a large excursion in phase space before it eventually returns to the stable steady state. This highly nonlinear response to perturbations is a direct consequence of our CAM model being an excitable system for this specific temperature regime. It means that small deviations from the fixed point can elicit large, transient orbits in phase space, seen as spikes in the time-signal. Thus, external noise driving our model can result in irregular patterns of simulated CO_2 uptake.

The constructive role of noise is further investigated in figure 3. Here, the CO_2 exchange in continuous light at a

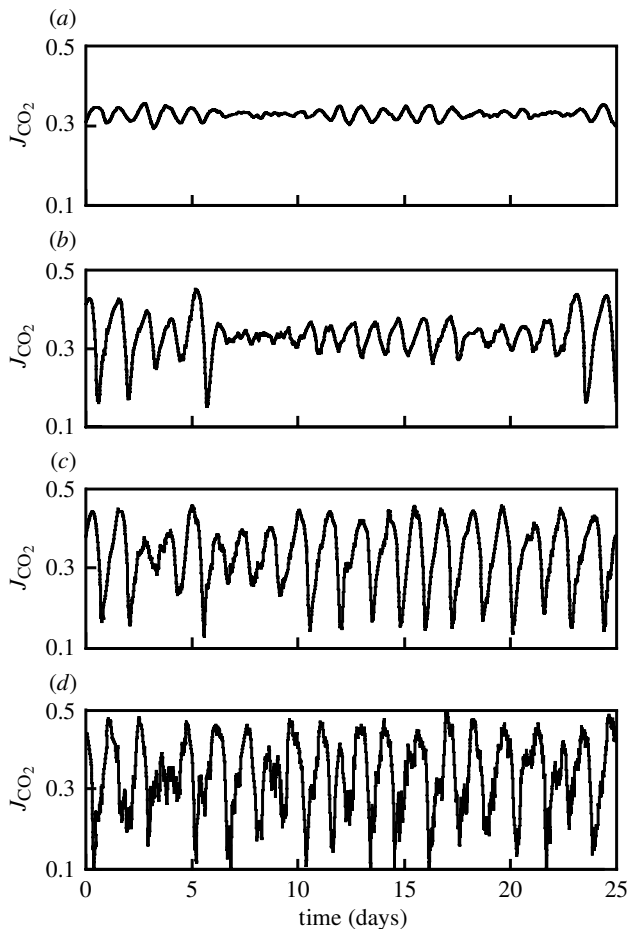


Figure 3. CO₂ gas exchange curves in continuous light for increasing noise strength R and at high relative temperature $T = 0.2255$. The curves go with increasing amplitudes from low fluctuations over almost regular oscillations for $R = 0.1$ to irregular time-structure for $R = 0.3$. This shows the effect of ‘stochastic coherence’ (Pikovsky & Kurths 1997). (Parameters as before.) (a) $R = 0.02$, (b) $R = 0.05$, (c) $R = 0.1$ and (d) $R = 0.3$.

relatively high temperature ($T = 0.2255$ relative units) is shown for different noise levels. Note that at this temperature without noise the system very quickly relaxes to a stable steady state (figure 2a). The addition of noise can change this behaviour dramatically. At the lower range of noise intensity only small fluctuations around the steady state are induced (figure 3a). If the noise amplitude is increased to intermediate levels, the first spikes are elicited (figure 3b). With further increase in noise, more and more spikes arise, until at an optimal noise intensity a regular pattern of maximal coherence is generated (figure 3c). Here, noise induces a coherent structure. The noise-excited oscillations are destroyed again at still larger amplitudes due to the destructive nature of noise (figure 3d). This effect of maximal enhancement at a certain noise level has been termed coherence resonance (Pikovsky & Kurths 1997), and it is characteristic for excitable systems, e.g. the FitzHugh–Nagumo model (FitzHugh 1961). To our knowledge, the phenomenon of noise enhanced oscillations is discussed here in our CAM model for the first time in relation to physiology of higher plants.

(a) CAM synchronization

Up to now we have treated the CAM cycle as if it were occurring in one single cell. In reality, however, a leaf consists of a large number of more or less strongly coupled CAM cells, and measurements, e.g. of CO₂ exchange, average over this ensemble. This has to be taken into account if noise has a strong influence, as shown before (figure 3). The timing for the occurrence of a spike is purely random. A second run using exactly the same external conditions will produce a rather different gas exchange pattern. Consequently, the random spikes will occur in different cells at different times, leading to a desynchronization between the individual cells of the leaf. Therefore, to compare results of our model in the presence of noise with experimental findings, we average CO₂ uptake, \bar{J}_{CO_2} , over an ensemble of CAM cells, each under the influence of its own noise representation, η_i , with common noise level, R :

$$\bar{J}_{\text{CO}_2} = \langle J_{\text{CO}_2}(\eta_i) \rangle. \quad (6)$$

The average extends over all cells of the ensemble, i.e. the whole leaf. Measurements have revealed a rather high diffusion resistance between leaf cells (Maxwell *et al.* 1997). This implies weak cell couplings. For simplicity and as a first approach, we restrict ourselves here to n uncoupled cells for which we perform the average.

Figure 4 compares measured CO₂ exchange time-series in continuous light with simulation results for the average over an ensemble of $n = 25$ uncoupled CAM cells under the influence of noise and for two different temperatures (scales in the simulation are in relative units). At $T = 20^\circ$ (figure 4a,b), starting from the same initial conditions, all cells are synchronized and the average shows a regular rhythm. Under the influence of noise the phases in the individual cells slowly drift apart. Subsequently, after some periods of nearly the same amplitude, with increasing desynchronization among the individual cells, the regular rhythm almost suddenly breaks down in the simulation (figure 4b). This same behaviour is observed in the experiments (figure 4a), and noise seems to be one possible candidate to explain this phenomenon. At the higher temperature, $T = 25^\circ$ (figure 4c,d) CAM is in the arrhythmic state, and this is well reproduced in the simulation in which the individual cells are stochastically fluctuating (see figure 3). As a consequence, different cells lose their phase information and become independent from each other.

Our explanation of the transition from accord to discord by phase desynchronization implies that it should also be possible to drive the system in the opposite direction through external synchronization of the cells, i.e. from discord (arrhythmic state) to accord (regular periodic state). Such transitions are indeed observed experimentally by fast change from high to low temperature (Grams *et al.* 1996; in this paper the existence of the reverse transition from disorder to order is shown for the first time, to the authors’ knowledge). As a further test for the phase desynchronization hypothesis, experiments with slow changes of temperature have been designed by Rascher *et al.* 1998 (figure 5a). Starting at high temperature, CAM is in the arrhythmic state. If the temperature is reduced quickly, regular oscillations are induced, as

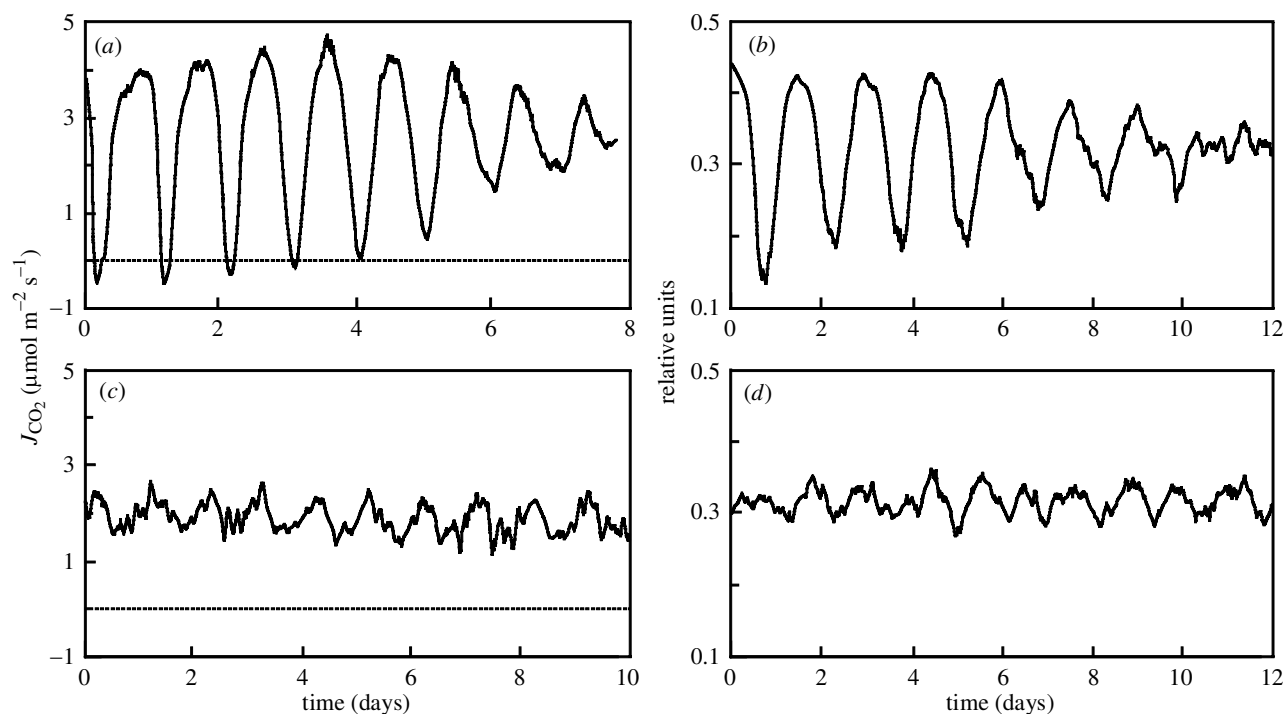


Figure 4. Experimental global CO_2 gas exchange curves (*a,c*) in comparison with simulated averages over an arrangement of 25 uncoupled CAM cells including noise in continuous light (*b,d*). At temperature $T = 20^\circ$ (*a*) the endogenous rhythm is well developed but dies out on the average because of increasing cell desynchronization. At the high temperature of $T = 25^\circ$ the individual cells already fluctuate stochastically between the fixed point and nearby limit cycles (see figure 2) which leads to the irregular time structure, observed globally in the experiment (*c*) as well as in the simulation (*d*). (Noise strength $R = 0.3$, other parameters as before.) (*a*) $T = 20^\circ$, (*c*) $T = 25^\circ$, (*b*) $T = 0.2250$ and (*d*) $T = 0.2254$.

observed earlier. Conversely, if the temperature is reduced slowly, as was done here, the integral over all cells remains irregular because the cells are still desynchronized. After only a short temperature pulse (applied at the end of day 7) the cells will be synchronized, and the rhythm is reinitiated by phase resetting (Winfree 1990, Murray 1993). The collective behaviour of an ensemble of cells leads to a memory effect: at intermediate temperature the whole plant leaf can show rhythmic or arrhythmic behaviour, depending on its history. In contrast to slow temperature reduction, slow increase of temperature does not lead to such hysteresis (Rascher *et al.* 1998). Independent of the rising time, rhythmicity is always inhibited at high temperature. These findings are remarkably well reproduced in the simulation (figure 5*b*), and this gives excellent support to our model assumption about the influence of noise on the CAM rhythm. In fact, these observations were predicted theoretically before the experiments were performed (Blasius 1997). They provide a new instance of understanding circadian rhythms as spatio-temporal patterns rather than on the time-scale alone (Winfree 1990, Achermann & Kunz, 1999).

4. CONCLUSIONS

Endogenous CAM rhythmicity has been investigated for many years, and is thus a very well-documented example of a free-running biological clock. Recent studies of the dependence of CO_2 gas exchange time-series on the external control parameters temperature and light intensity have shown that a small change in the para-

meters can bring about a sudden breakdown of rhythmicity (Lüttge & Beck 1992; Grams *et al.* 1996, 1997). Time-series analysis has indicated that this is not a transition to complete stochasticity, but that elements of regular dynamics are retained (Lüttge & Beck 1992; Blasius 1997). Moreover, rhythmicity to arrhythmicity transitions have been shown to be reversible, which means that arrhythmicity is a state of CAM and not an irreversible transformation in the cell structure (Grams *et al.* 1997). However, neither from the time-series analysis nor from model analysis has it been possible thus far to interpret the irregular behaviour as a clear transition to deterministic chaos.

Realizing that noise is unavoidable in all living structures, and that in a highly nonlinear dynamic system, such as the CAM cycle, stable and unstable areas in phase space normally exist in close proximity, we investigate in this paper the influence of stochastic noise on CAM dynamics. Employing a minimal model which contains all the essential features of the biophysical cell structure of CAM plants, and which lends itself to a clear fixed point analysis, we found a set of limit cycles close to the high temperature fixed point, which is typical for the structure of excitable systems. Because of this, noise can act constructively in the form of coherence resonance. For the single cell this implies, dependent on the noise level and thus on temperature, a resonant-like sequence of irregular and regular time-patterns.

Integral gas exchange measurements always imply an average over a large number of CAM cells in the leaf. Performing simulations and averaging over an ensemble of independent CAM cells predicted an increasing

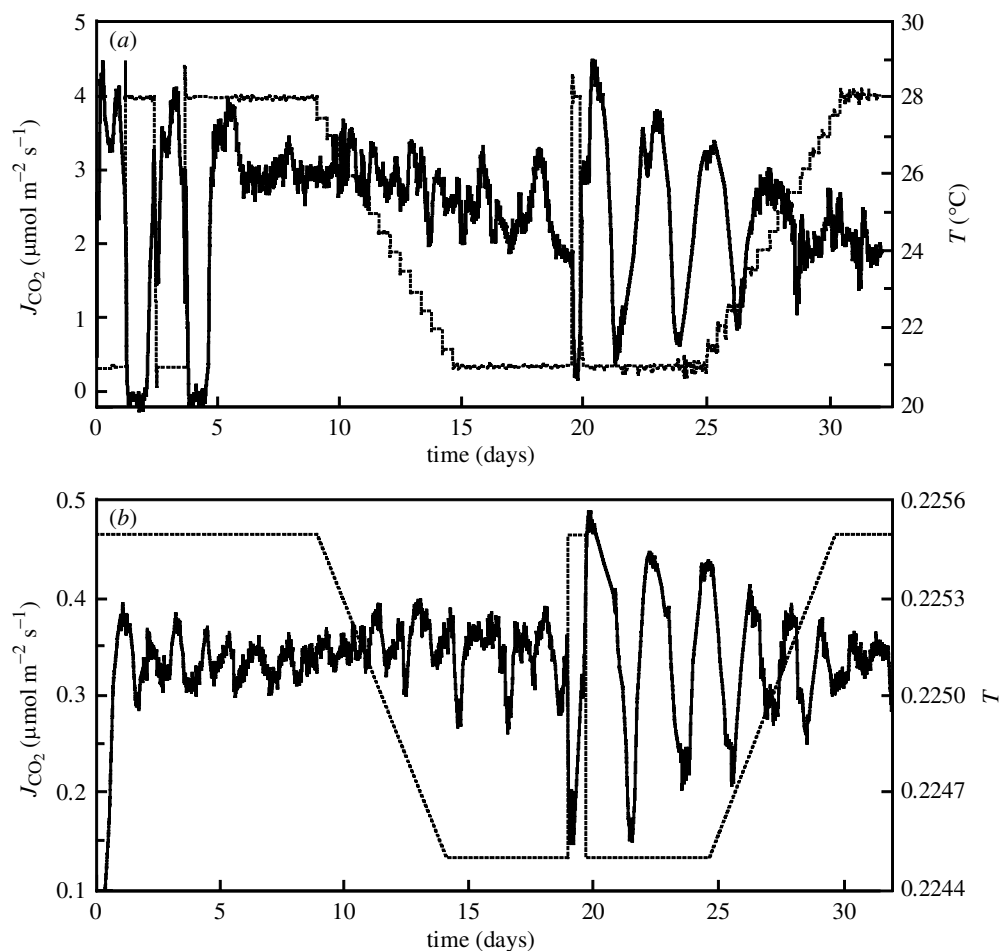


Figure 5. (a) Experimental results in CO_2 exchange for slow decrease of temperature from high value $T = 28^{\circ}\text{C}$ (irregular regime) to low value $T = 21^{\circ}\text{C}$ (normally regular regime) (Rascher *et al.* 1998), in comparison with (b) simulation results averaged over 25 cells. (The scales in the simulation are in relative units, the low temperature corresponds to the stable limit cycle $T = 0.2245$ in figure 2; $R = 0.3$, other parameters as before.)

dephasing of the individual rhythms. Corresponding experiments and their simulation lead to an intriguing explanation of arrhythmicity, occurring in narrow intervals of temperature or photon flux density: stochastic excitations generate phase desynchronization of individual cells in the leaf. These results are relatively independent of the specialities of the CAM model employed for the simulations. They rather depend on the general dynamic structure of excitable systems. In this sense our results are generic for the functioning of biological clocks, and not merely a peculiarity of photosynthesis oscillations.

These results relate the time-structure exhibited by the intact leaf of a CAM plant to the spatial structure of synchronization at the level of individual cells. In a living plant the presence of noise under continuous illumination would thus lead to leaf patchiness of photosynthesis. Indeed, the first experimental findings (Rascher *et al.* 2001) using non-destructive chlorophyll-fluorescence imaging (for an outline of this technique see Osmond *et al.* (1998, 1999)) support this new hypothesis.

ENDNOTE

¹We use here the scaled equations with non-dimensional quantities which reduce the number of free parameters

to the limited set essential for the correct dynamical structure. For comparison with experimental results, the model has to be rescaled with parameters adjusted to physiological values. The procedure is described extensively in Blasius *et al.* (1999).

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