TRANSIENT SYNCHRONISATION IN YEAST CELL POPULATIONS OF INTERMEDIATE DENSITY

André Weber
Special Laboratory for Electron and Laserscanning Microscopy
Leibniz Institute for Neurobiology Magdeburg
Germany
andre.weber@lin-magdeburg.de

Werner Zuschratter
Special Laboratory for Electron and Laserscanning Microscopy
Leibniz Institute for Neurobiology Magdeburg
Germany
zuschratter@lin-magdeburg.de

Marcus J. B. Hauser
Institute for Biometry and Medical Informatics
Otto-von-Guericke-Universität Magdeburg
Germany
marcus.hauser@ovgu.de

Abstract
The mechanism of the transition between synchronised and desynchronised behaviour of intact yeast cells of the strain *Saccharomyces carlsbergenis* was investigated. In cell colonies of intermediate cell density, all cells remain oscillatory, in addition, a partially synchronised and a desynchronised state are accessible for experimental studies. In the partially synchronised state, the mean oscillatory frequency is shorter than that of the cells in a desynchronised state, showing phase advancement. The cells do not synchronise completely, as the distribution of the oscillatory frequencies only narrows but does not collapse to a single frequency. The desynchronisation is characterised by a broadening of the distribution of oscillation frequencies of the cells. Chimera states could not be observed.

Key words
Synchronisation, glycolysis, yeast cells, phase advancement, coupled oscillators.

1 Introduction

Synchronisation is a frequent manifestation of self-organisation, where individuals coordinate their behaviour in time, leading to the emergence of collective, macroscopic dynamics [Winfree, 2001; Kim et al., 2007; Stark et al., 2007]. For instance, in synchronised populations of cells, all individuals adhere to a collective, common rhythm. This is also the case during glycolytic oscillations of yeast cells [Richard, 2003]. Although the physiological function of glycolytic oscillations in yeast cells is not yet known [Chandra et al., 2011], it is conjectured that for unicellular organisms, such as yeast, life within a community is beneficial for their long-term survival [Palková and Váchová, 2006]. Efficient cell-to-cell communication is a prerequisite for the organisation of communities, and such communication among individuals may be provided by metabolic oscillations.

Glycolysis is a fundamental pathway in the energy metabolism of eukaryotic cells and it may show oscillatory dynamics. During glycolytic oscillations of yeast cells, the coupling among individual cells is based on the relay and subsequent diffusion of the messenger molecule acetaldehyde [Richard et al., 1996] through the extracellular medium, where it is absorbed by the other cells. When a sufficient concentration of this molecule is reached, the cells synchronise their rhythm to that of the messenger-rich extracellular medium.

The dynamics of a cell population depends on cell density. At high densities yeast shows synchronous, coherent oscillations. Once the cell density falls below a critical threshold, the yeast population no longer displays any collective oscillations, but rather remains quiescent [Aldridge and Pye, 1976]. For populations of immobilised yeast cells, the transition between collective oscillatory and quiescent dynamics at the population level follows a Kuramoto transition [Pikovsky et al., 2004; Strogatz, 2000]: The collective oscillatory signal is generated at high cell densities, where the oscillations of all individual cells are synchronised both in phase and frequency. At low densities, the individual cells remain oscillatory, but they lose their phase and frequency coherence so that each of them oscillates with its own phase. As the collective behaviour

is the sum of the signals of the individual cells, phase-incoherent oscillations will lead to stationary, quiescent collective dynamics [Kuramoto, 1975; Shinomoto and Kuramoto, 1986]. Early reports that individual cells remain oscillatory in unstirred cell suspensions at low cell densities [Aon et al., 1992] have recently been confirmed in experiments using either *S. carlsbergensis* cells immobilised on coverslips [Weber et al., 2012] or *S. cerevisiae* fixed in a microfluidic device [Gustavsson et al., 2012].

The present study aims at investigating the mechanisms leading to the synchronisation (desynchronisation) of cells in a yeast population. At intermediate cell densities, it takes some time until partial synchronisation can be achieved, such that both the synchronised and desynchronised states are easily accessible in experiments.

2 Materials and methods

Cells of the yeast *Saccharomyces carlsbergensis* were cultivated, harvested, washed and stored as described by [Weber et al., 2012]. 100 μl of cell suspension containing different cell densities were prepared and transferred to the batch chamber (for details, see [Weber et al., 2012]). There, the cells sedimented and were immobilised at the poly-D-lysine-coated coverslip. Then the experiment was started by adding 3 mmol/l KCN to induce anaerobiosis by blocking the activity of the mitochondria. About 10 min later, 52 mmol/l glucose were added to trigger the glycolysis and induce the metabolic oscillations in the cells.

The intracellular dynamics was monitored through the autofluorescence of NADH which serves as an indicator for glycolytic activity [Hess and Boiteux, 1968]. The NADH autofluorescence from single yeast cells was measured with an inverted microscope equipped with a position-sensitive single photon counting photomultiplier tube [Prokazov et al., 2014; Vitali et al., 2011]. For excitation of intracellular NADH a 8 MHz pulsed frequency-tripled Nd:vanadate laser tuned at 355 nm was used. The emission light of yeast cells was filtered by both a long-pass and a bandpass filter and detected by the photomultiplier. The positions where the incident photons were detected on the photomultiplier were binned into frames of 512×512 pixels, resulting in a resolution of 0.33 μm/pixel in the object space. The field of view had a diameter of 169 μm. A time binning of 2s was chosen as being appropriate to analyse glycolytic oscillations.

The immobilised yeast cells were randomly distributed on the coverslip. We have analysed the NADH fluorescence signal originating from each of the cells as well as the collective population signal. The fluorescence signal from each immobilised cell was monitored in time. At any instant, the single cell fluorescence F_{cell} is the mean value of the intensity of the fluorescence signal detected in the area occupied by an individual cell, $F_{cell} = a^{-1} \sum c$, where c is the number of incident photons originating from the area a occupied by the individual cell. The temporal sequence $F_{cell}(t)$ yields the time evolution of the fluorescence of an individual cell.

The time-series of the fluorescence of each cell was subjected to a baseline subtraction such that any spurious drifts or long-term trends were eliminated. The frequencies of oscillations were obtained by a fast Fourier transform of the time series. These baseline subtracted single cell fluorescence signals were averaged to obtain the collective signal of the population.

Noise reduction was achieved by bandpass filtering. The applied Fourier bandpass filter cut off frequencies higher than 0.05 Hz and lower than 0.014 Hz. Thus, the frequencies of the glycolytic oscillations were preserved in the filtered time series $x_i(t)$.

The phase $\phi_i(t)$ of each oscillating cell i

$$\phi_i(t) = arctan\left(\frac{\widetilde{x}_i(t)}{x_i(t)}\right) \tag{1}$$

was computed through the Hilbert transform

$$\widetilde{x}_i(t) = \frac{1}{\pi}\int_{-\infty}^{\infty} \frac{x_i(t')}{t-t'}dt' \tag{2}$$

of the filtered single cell signal $x_i(t)$. The macroscopic oscillation phase Φ is obtained as the average phase of all oscillating cells

$$\Phi(t) = N^{-1}\sum_{i}^{N} \phi_i(t). \tag{3}$$

The order parameter K [Shinomoto and Kuramoto, 1986],

$$K(t) = \left| N^{-1}\sum_{i}^{N} e^{i\phi_i(t)} - \left\langle N^{-1}\sum_{i}^{N} e^{i\phi_i(t)} \right\rangle \right|, \tag{4}$$

was chosen for measuring the phase synchronisation at each time point. An order parameter K = 1 indicates a complete synchronisation among the cells in a population, whereas K = 0 means that the cells oscillate at random phases.

3 Results

Intact yeast cells of the strain *S. carlsbergensis* reveal that the dynamics of glycolytic oscillations depends on the cell density of the population. Whereas at cell densities $\rho > 0.3\%$ all cells synchronise their metabolism to a joint rhythm, such a synchronisation can no longer be attained for populations of cell densities $\rho < 0.01\%$. We have studied the behaviour of cell populations at intermediate cell densities, i.e., at $0.001\% \leq \rho \leq 0.3\%$, where the cells are capable to synchronise transiently.

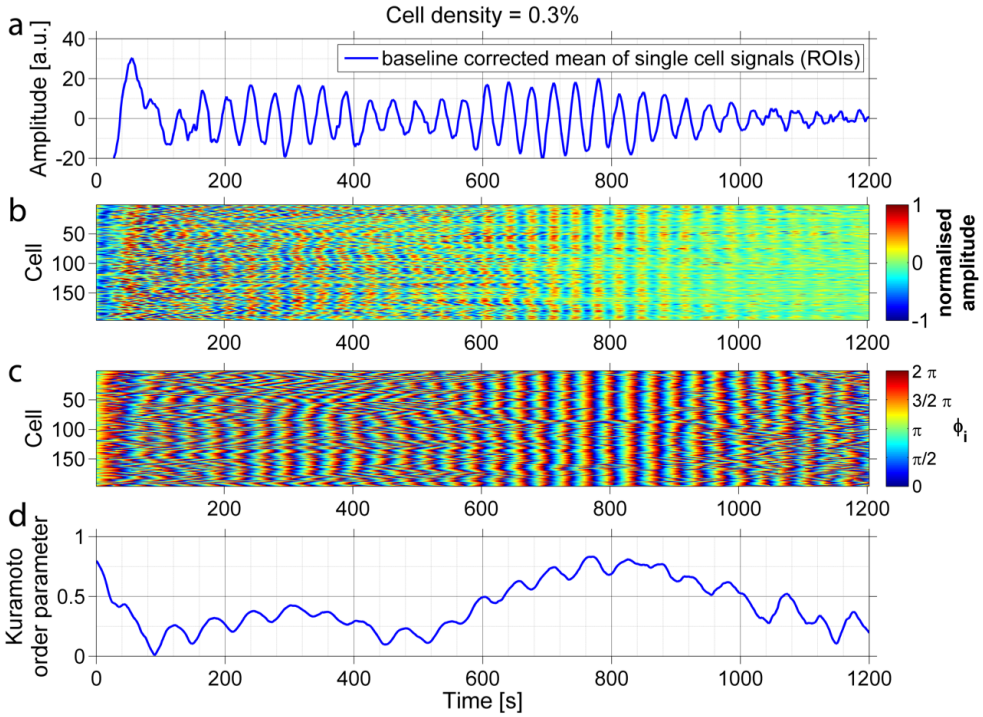

Figure 1. Collective and individual dynamics of *S. carlsbergensis* at a cell density of 0.3% shows a transition from partially synchronous to desynchronous dynamics. Oscillations start at $t = 0$ s about 200 s after addition of glucose. (*a*) The time-series of the collective fluorescence signal oscillates with changing amplitudes. Two episodes of oscillations of larger amplitude occur, the first between 200 s $\leq t \leq$ 400 s and the second between 600 s $\leq t \leq$ 800 s. (*b*) The individual cells remain oscillatory during the experiment. At $t = 600$ s a transient episode of synchronised collective oscillations sets in and the population signal attains its highest amplitudes. For $t \geq 1000$ s the single cell oscillation amplitudes dampen, which causes a decaying population signal. (*c*) At the begin and the end of the experiment most of the cells oscillate at their own periods and phases (ϕ_i), which leads to lower amplitudes in the collective signal (*a*). The synchronisation episode at 600 s $\leq t \leq$ 1000 s is caused by a temporary entrainment of the oscillations of the individual cells. (*d*) The time-dependent order parameter K reflects the degree of synchronisation among the cells.

Glycolytic oscillations were induced by the addition of an aliquot of glucose to the cell medium containing starved yeast cells. By convention, time $t = 0$ s denotes the onset of the glycolytic oscillations approximately 40 - 200 s after glucose addition. The oscillatory response of the yeast cells was long-lasting, however, always transient, since the experiments were performed under batch conditions. In fact, the population signals of intermediate density are well detectable (Fig. 1) and show that oscillations decay at long times (i.e., at $t >$ 1000 s in Fig. 1a).

In a population of cell density ρ = 0.3%, desynchronisation begins to set in at $t >$ 1000 s (Fig. 1). It manifests itself in macroscopic oscillations whose amplitudes may change in time (Fig. 1a). On the other hand, on the level of individual cells, each cell shows glycolytic oscillations. However, for a long time (i.e., up to $t \sim$ 550 s) these oscillations are desynchronised in both amplitude (Fig. 1b) and phase (Fig. 1c). After this induction time, the amplitude of collective oscillations increased (Fig. 1a), indicating the formation of a transient episode of large-amplitude oscillations. These are due to an intermittent synchronisation of the oscillations of the individual cells (e.g., from 600 s $\leq t \leq$ 1000 s in Fig. 1b,c). The time-dependent order parameter K reflects this behaviour: it lies between 0.2 and 0.4 for times $t \leq$ 550 s indicating that the oscillation phases of the cells are desynchronised (Fig. 1d). For $t \geq$ 600 s cells partially synchronised their oscillations for about 400 s and K reached values higher than 0.75.

The distribution of the oscillation periods of the individual cells was determined for the two episodes of distinct macroscsopic oscillations shown in Fig. 1. The distribution of the oscillation periods during desynchronised oscillations (that lasts from $\approx$ 150 s $\leq t \leq$ 450 s, Fig. 1) is relatively broad (Fig. 2), whereas the distribution narrows considerably during the episode of partially synchronous oscillations (from $\approx$ 600 s $\leq t \leq$ 1000 s, Fig. 1). However, the cells do not achieve a complete synchronisation, since the frequency distribution is not completely suppressed. Furthermore, we observe that the synchronised oscillations present a shorter mean period (T = 33 s) than that of the desynchronised oscillations, whose mean period is about T = 37 s (Fig. 2). Thus, the cells synchronise by tuning their frequencies to a slightly higher common frequency. This means that a considerable fraction of the cells advance their phases in order to become synchro-

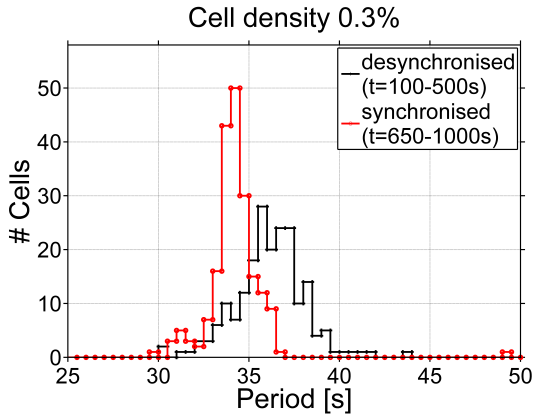

Figure 2. Histograms of oscillation periods of individual cells in a population of intermediate cell density (ρ = 0.3%) for episodes of desynchronised (black line) and partially synchronised (red line) oscillations. During desynchronisation (100 s $\leq t \leq$ 500 s, Fig. 1) the oscillations of the cells show a fairly broad distribution of oscillation periods. In the course of partial phase synchronisation (650 s $\leq t \leq$ 1000 s, Fig. 1) the oscillation periods decrease and the distribution of periods narrows since most cells entrain their oscillations to a common frequency (period).

nised.

The behaviour of the phases of the glycolytic oscillations (Fig. 3c) accompany that of the oscillatory frequencies (Fig. 3b). As the cells synchronise, the initially random phase distribution narrows, and during the synchronisation events the phase distribution is characterised by a pronounced maximum. The temporal evolution of the oscillatory periods and of the phase difference $\phi_i - \Phi$ between the phase ϕ_i of each individual cell to that of the mean phase Φ of all cells is plotted for a cell population of density ρ = 0.1% (Fig. 3). Note that the distribution of the phase differences $\phi_i - \Phi$ narrows visibly as the cells synchronise.

During the partial synchronisation events of glycoyltic oscillations, the cells present a narrow but continuous distribution of oscillatory frequencies and phases. Once a substantial fraction of the nutrient (glucose) is consumed by the cells, the amplitudes of the oscillations of the individual cells decrease (at $t >$ 800 s in Fig. 1b and at $t >$ 1100 s in Fig. 3). The cells start releasing smaller concentrations of the messenger molecule to the environment, and hence, the coupling between the cells weakens. This leads to a broadening of the distributions of both, cell periods and cell frequencies. Consequently, the global fluorescence signal decays until it becomes quiescent, whereas the individual cells continue oscillating each at their own, individual pace.

4 Discussion

We have studied details of the mechanism leading to synchronisation and desynchronisation of the glycolytic oscillations in immobilised yeast cells.

The coupling between cells is provided by the messenger molecule acetaldehyde. During glycolytic oscillations the cells release acetaldehyde periodically to the extracellular medium, where the concentration of this messenger is detected by other cells. Whence and where the extracellular concentration of acetaldehyde is sufficiently high, yeast cells start to oscillate in synchrony with the oscillating concentration of acetaldehyde in the extracellular medium. The magnitude of acetaldehyde oscillations hence provides for the strength of the coupling between the cells of a population. At intermediate cell densities (0.01 $< \rho <$ 0.3%), both, the synchronised and the desynchronised cells are available for experimental studies, since the transition from desynchronisation to (partial) synchronisation takes sufficiently long.

At intermediate cell densities the cells slowly synchronise their rhythms, and after a while (e.g., at about t = 550 s, Fig. 1) the extracellular concentration of acetaldehyde becomes coherent in time and space. Once this is achieved, we observe an episode of relatively high synchronisation among the cells of the population (in the interval 550 s $\leq t \leq$ 1000 s, Fig. 1). It is noteworthy that amplitudes of the oscillations of the single cells have already begun to decay as the macroscopic collective signal attains its highest amplitudes.

The synchronisation of the cells is not complete, as evidenced from the distributions of the oscillatory frequencies (periods, Figs. 2 and 3c) and phases (Figs. 1c and 3b) instead of showing a single, unique frequency. During the episodes of desynchronised oscillations the variability in oscillation period was larger than at times where the cellular oscillations are synchronised (Fig. 2). Furthermore, the synchronisation of cells led to a slightly shorter mean period of the intracellular glycolytic oscillations as compared to the periods of oscillations when the cells were desynchronised (Fig. 2). Hence, events of synchrony are caused by the entrainment of cells to a period that is slightly shorter than their free-running oscillatory periods. Thus, the synchronisation takes place by locking many cells to a slightly faster rhythm, in other words, by advancing the phase of many oscillators (cells).

Desynchronisation of such incompletely synchronised cell populations sets in when the nutrient glucose is partially consumed by the cells. Then, only lower concentrations of the messenger molecule can be released to the extracellular medium, thus decreasing the coupling strength in the medium. We observe that the distributions of both frequencies and phases broaden. Whereas cells continue to oscillate, the loss of frequency and phase coherence leads to a quiescent population signal.

During the partial synchronisation events of glycoyltic oscillations, the cells present a continuous distribution of oscillatory frequencies and phases. In fact, we cannot distinguish between two dynamic subpopulations, where one oscillates synchronously whether the other remains either quiescent or oscillates at a distinctively

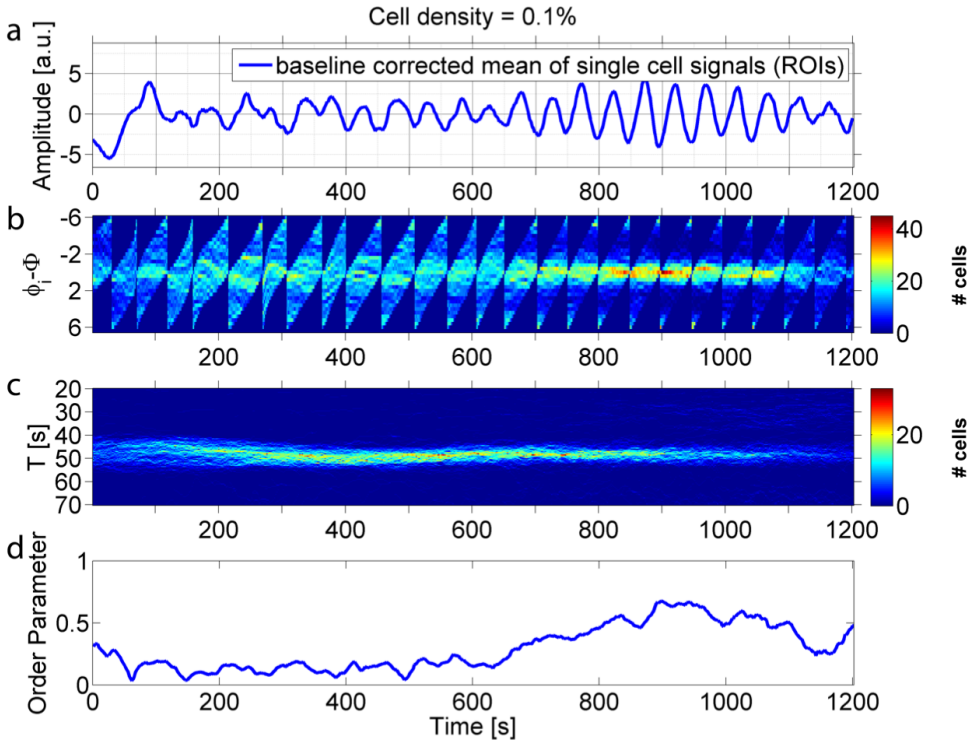

Figure 3. (*a*) The time-series of the collective fluorescence signal for a time series of cell density $\rho = 0.1\%$. Partial synchronisation of cellular oscillations occurs at $650\,\mathrm{s} \leq t \leq 1100\,\mathrm{s}$. (*b*) Phase difference $\phi_i - \Phi$ between the phase ϕ_i of each individual cell to that of the average phase Φ of all cells of the population. Note that the phase differences diminish drastically during the episode of synchronisation. (*c*) Temporal evolution of the periods T of the oscillations of the cells. (*d*) The time-dependent order parameter K.

different frequency (or phase). Thus, we believe that yeast cell populations performing glycolytic oscillations are unable to form chimera states. This seems also to be in line with the accepted coupling mechanism between the cells, which is due to the release and sensing of a messenger species. Thus, the coupling in cell populations, at least at intermediate and low cell densities, is rather local than global.

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