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Antispirals in an artificial tissue of oscillatory cells

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In a tissue of oscillatory cells the active intracellular medium is surrounded by a membrane and the cells are separated by inactive extracellular medium. The synchronization properties of a system of such coupled oscillatory cells have been emulated using the light-sensitive Belousov-Zhabotinsky reaction. Experimental results for four coupled cells are confirmed by numerical simulations. We have furthermore demonstrated the existence of antispirals and antipacemaker waves with inward propagating waves in larger cell assemblies of this type. Such dynamical structures are extremely rare in homogeneous chemical systems where the generic behavior is normal spirals and target patterns with outward-moving waves.

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Models of coupled oscillatory cells in biological systems have thus far been proposed with the assumption that the concentrations of oscillatory species are homogeneous inside each cell [1–3]. Concentration gradients observed in connection with fast NADH oscillations in neutrophils [4] suggest the use of reaction-diffusion equations to model the intracellular as well as the extracellular concentrations. In an assembly of oscillatory cells, e.g., a tissue, the oscillatory medium is contained in cells and is surrounded by a cell membrane and inactive extracellular medium. In this study we have investigated, experimentally and numerically, the effect of nonhomogeneous, small-amplitude oscillations in interacting cells by using the light-sensitive Belousov-Zhabotinsky (BZ) reaction as the oscillatory medium. The oscillatory properties of the regions between the ''cells'' were suppressed by application of a sufficient intensity of light. Small-amplitude oscillations such as the ones studied here occur naturally in yeast cells [5,6].

Here, we describe how an assembly of cells containing an active, oscillatory medium, separated by an inactive extracellular medium, displays significantly different behavior from that of a normal oscillatory medium not containing inactive parts. To model the cell assembly we use the dimensionless Oregonator model [7] with diffusion terms, which is a qualitatively good model for the BZ reaction [8],

$$\frac{\partial u}{\partial \tau} = \frac{1}{\varepsilon}\{qw - uw + u - u^2\} + \nabla^2 u,$$

$$\frac{\partial v}{\partial \tau} = u - v,$$

$$\frac{\partial w}{\partial \tau} = \frac{1}{\varepsilon'}\{\varphi(\mathbf{r}) - qw - uw + 2fv\} + \nabla^2 w. \qquad (1)$$

The variables u, v, and w represent the dimensionless concentrations of the chemical species $HBrO_2$, $Ru(bpy)_3^{3+}$, and Br^-, respectively, and ε, ε', q, and f are dimensionless parameters. The scaling of the dimensionless time τ and the dimensionless length r is chosen such that the diffusion constants of u and w (assumed equal in aqueous solution) can be set to 1. In the experiments with the BZ reaction the catalyst $Ru(bpy)_3^{3+}$ is immobilized in a thin, quasi-two-dimensional layer of silica gel. To correspond as well as possible to these experiments, the diffusion constant of v is set to zero and the simulations are carried out for two spatial dimensions. The term φ represents the photoinduced Br^- production proportional to the light intensity of the perturbation light used in experiments [9,10]. In the simulations this term is made spatially dependent, i.e., depending on the position in the system.

In Table I are shown the two sets of parameter values applied in this study, with ε, ε', q, and f being equal for both sets, but with φ being different in the sets labeled ''intracellular'' and ''extracellular.'' The intracellular parameter values yield small-amplitude oscillations, close to a supercritical Hopf bifurcation, whereas the extracellular parameter values correspond to a stable stationary state. Figure 1(a) shows a snapshot of a simulation of a single cell. The simulation was done on a 100×100 grid with a grid size of 0.24 on the dimensionless length scale. Intracellular parameter values were used in the central 70×70 grid points, having extracellular parameter values in the rest of the system. The snapshot shows a clear difference between the value of v in the intracellular and extracellular media (see color code for values of v). Figure 1 (b) shows the profiles of v at three different time instants through the middle of the cell [marked by the dashed white line in (a)]. There are three important features to notice regarding the profiles. The intracellular medium is inhomogeneous across the cell with a convex distribution of v, and it has a sharp but continuous concentration change across the cell membrane (this is the term chosen for the interface between the active and inactive parts of the sys-

TABLE I. Parameter values for the intracellular and extracellular media.

Parameter	Intracellular	Extracellular
ε	0.45	0.45
ε'	0.0009	0.0009
q	0.000 95	0.00095
f	0.79	0.79
φ	0	0.001

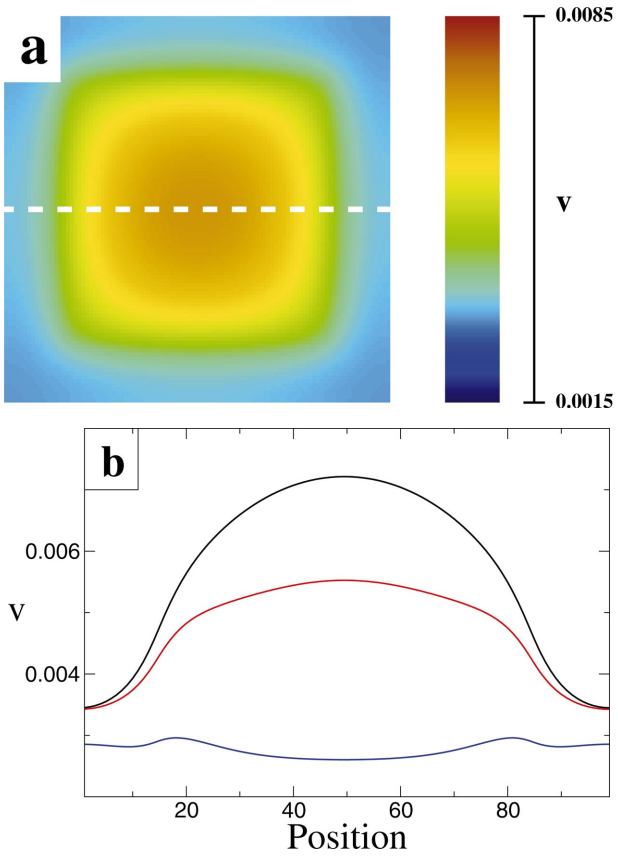

FIG. 1. (Color) Properties of a single cell: (a) a snapshot at a certain time instant with the color scale as indicated, (b) profiles through the middle (marked by the dashed white line), also at different instants.

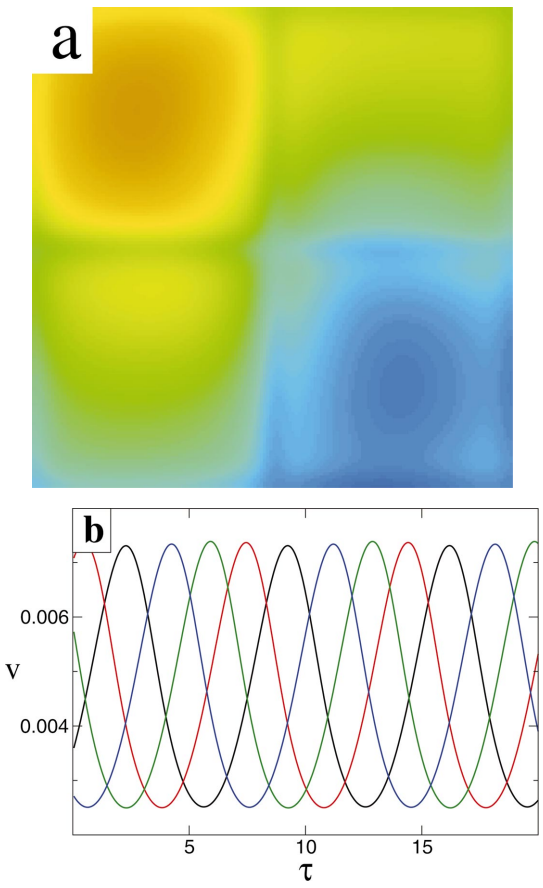

FIG. 2. (Color) Synchronization in antiphase of 2×2 cells: (a) a snapshot of the cells, (b) the time series of the centers of the four cells. The color scale is shown in Fig. 1.

tem). Third, the oscillations in the passive extracellular medium are driven by the intracellular oscillations. Oscillating yeast cells have similar features regarding the acetaldehyde (ACA) concentration, and the interplay between the intracellular and extracellular media has been studied in a stirred suspension of cells performing small-amplitude oscillations. In that system it was shown that intracellular ACA oscillations can drive extracellular ACA oscillations to mediate synchronization between cells [5,11]. The difference between intracellular and extracellular ACA concentrations implies that an ACA gradient must exist across the cell membrane and that the intracellular concentration distribution is not homogeneous. Cells constructed with slightly different sizes (e.g., 60×70 grid points) have slightly different eigenfrequencies.

To emulate a tissue, four such cells were arranged in a 2×2 array with the cells separated by extracellular medium, six grid points wide. After a while the oscillations of the centers of the four cells were synchronized either in-phase or antiphase *depending on initial conditions*. By in-phase is meant that the four centers all oscillate with the same frequency and that they are all in the same phase of the oscillations. By antiphase is meant that they oscillate with the same frequency but the two nearest neighbors are 90° out of phase. In Fig. 2(a) is shown a snapshot of four cells synchronizing in antiphase, and in (b) is shown the corresponding time series for the centers of the four cells.

A majority of initial conditions lead to in-phase synchronization. If the cells are constructed with slightly different sizes and eigenfrequencies they synchronize almost in-phase (with a narrow distribution of oscillation phases) regardless of initial conditions. This leads us to believe that only in-phase (or almost in-phase) synchronization is observable in experiments.

To study the behavior of larger arrays of cells, simulations with 8×8 cells, each cell having the size of 70×70 grid points, were performed. As before, the cell-cell distance is six grid points. There are, again, two main results of the simulations. The cells couple to yield an antispiral, i.e., the spiral arms move inwards, or an antipacemaker pattern, i.e., wave fronts move inwards. Which pattern is selected *depends on initial conditions*. Figure 3 shows four consecutive snapshots of [(a)–(d)] an antipacemaker, and [(e)–(h)] an antispiral pattern. In these simulations a thick edge of inactive medium has been put around the 8×8 cells to emphasize the nature of the two types of patterns (smaller edge leads to a larger wavelength). Control simulations with cells of different sizes lead to the same results, indicating robustness of the results. Doing a similar simulation with periodic boundary conditions leads for suitable initial conditions to the formation of a plane wave moving through the system. We thus expect that the phenomena observed for 8×8 cells

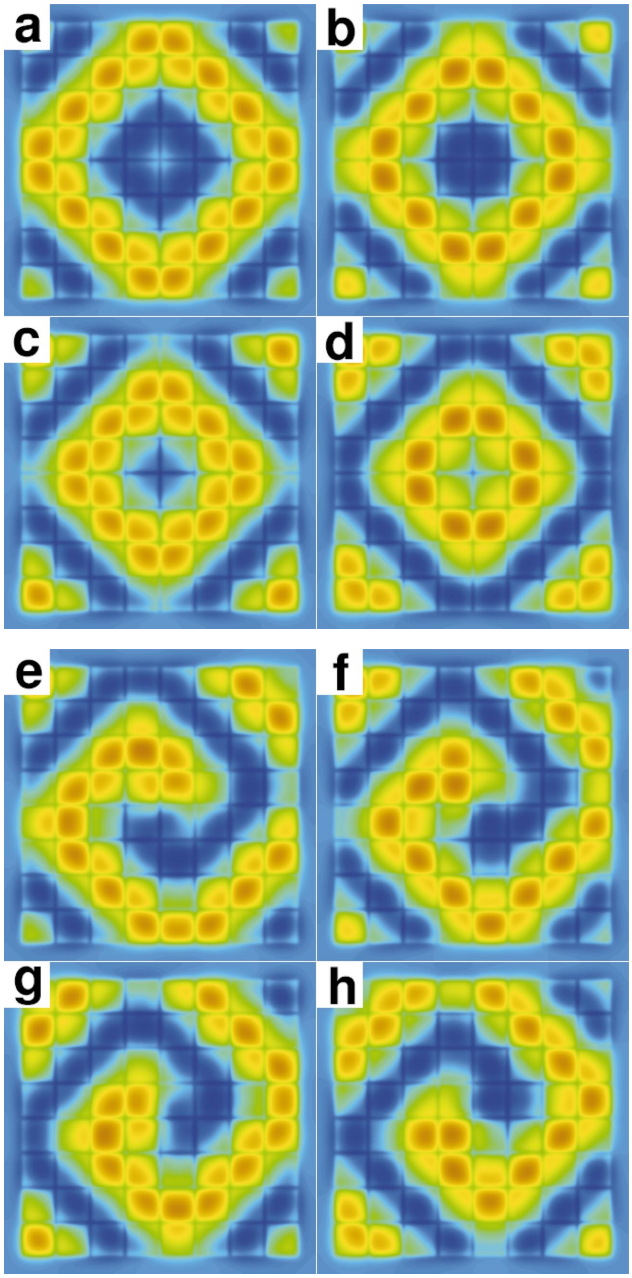

FIG. 3. (Color) Succesive snapshots of antipacemaker (a–d) and antispiral patterns (e–h) formed in an assembly of 8×8 cells, with $\approx T/8$ between each snapshot (T being one oscillation period). Parameter values were the same for both simulations, the only difference was the initial conditions. The color scale is shown in Fig. 1.

would also be observed for even larger systems, since spirals and target patterns are merely plane waves sufficiently far from the center.

The in-phase synchronization observed in the 2×2 simulations is just a special case of the antipacemakers observed for larger systems. It only looks like in-phase synchronization when the observed points are selected at equal distance from the center of the two-dimensional system. The phase of a point at a larger distance from the system center is ahead of the phase of a point closer to the center. This phase dependence is equivalent to the inward-moving waves in larger systems. For a system of 3×3 cells (not shown here) this pattern is evident, and since 3 is an odd number the waves do not collide in the center of the system, which is the center of a cell, but at one of the corners of the central cell. The selected corner depends on initial conditions.

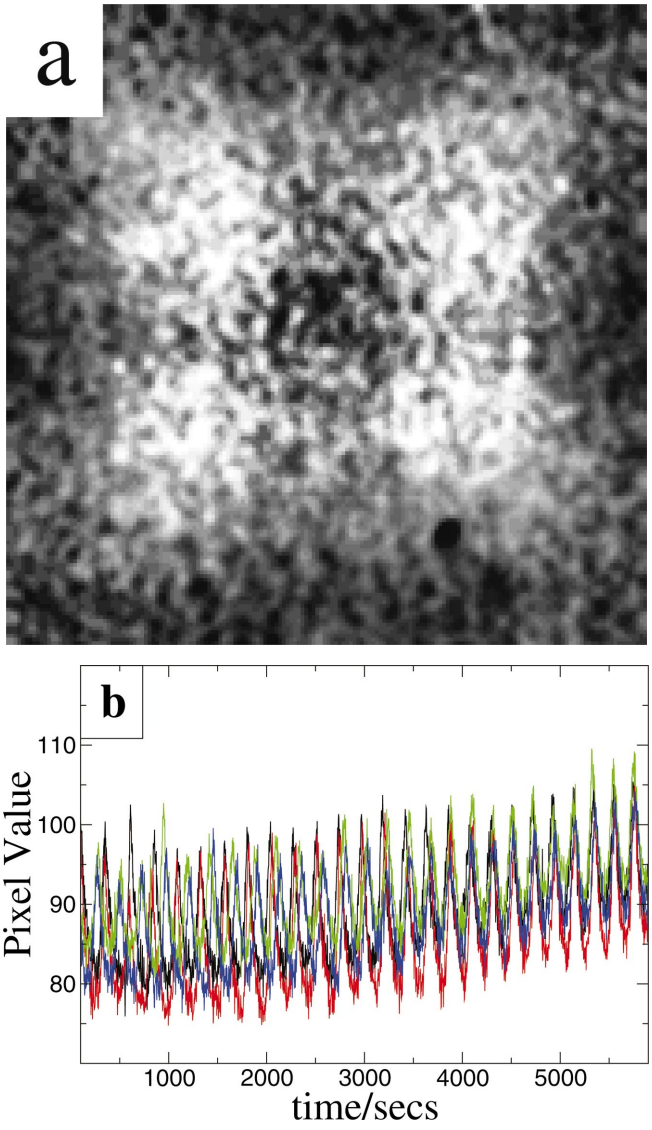

FIG. 4. (Color) Enhanced experimental image of in-phase synchronization (a), and the time series of the centers of the four cells (b). Initially they are out of phase, but eventually they synchronize in-phase.

If the cell-cell distance is increased for 8×8 cells, no ordered pattern such as antispirals or antipacemakers is observed. Each cell still responds to the state of its neighbors, but evidently it is not fast enough for the antispiral or antipacemaker to form. In none of the simulations in-phase oscillations across the entire system were observed.

When no light grid is imposed on the system, simulations with this model usually only result in normal spirals, i.e., outwardly moving spiral arms, and no target patterns. This is also true for experiments with the BZ reaction excluding target patterns resulting from external pacemakers [12–14].

Experiments were carried out with the light-sensitive BZ reaction. A gel containing the reduced form of the catalyst $Ru(bpy)_3^{2+}$ was prepared by acidifying a solution of 10% (w/w) Na_2SiO_3 and 2.0 mM $Ru(bpy)_3^{2+}$ with H_2SO_4 and casting a uniform 0.3 mm$\times$20 mm$\times$25 mm layer onto a microscopic slide. A solution not containing any catalyst was prepared by mixing bromate, sulfuric acid, and malonic acid solutions with the concentrations $[BrO_3^-]=0.013M$, $[H_2SO_4]=0.5M$, and $[MA]=0.5M$. These concentrations result in small-amplitude oscillations in the gel, to our knowledge the first known example of this in the light-sensitive BZ reaction-diffusion system. By continuously recycling the catalyst-free solution, in which the gel was immersed, having a total volume of $\approx$230 mL the system was kept semiopen. This volume was large enough compared to the volume of the gel to exclude effects relating to the system being closed. A light grid was focused on the gel by projecting light passed through a mask onto the surface of the gel using the appropriate lenses [19]. Figure 4(a) shows an enhanced (due to very low contrast) experimental snapshot of four cells synchronizing in phase, and in Fig. 4 (b) is shown the corresponding time series of the centers of the four cells. Initially they are out of phase but eventually synchronize (almost) in-phase. It was not possible to observe antiphase synchronization, in accordance with our expectations from numerical simulations. Limitations in the present setup prevented us from studying 8×8 cells experimentally.

We have demonstrated how an assembly of cells containing an active, oscillatory medium, separated by inactive extracellular medium, displays either antispirals or antipacemakers. A normal oscillatory medium not containing inactive parts shows different behavior from this, and in chemical systems in aqueous solution only outwardly moving spirals have been observed thus far. The same goes for models of these. Without changing diffusion coefficients, we have, by suppressing oscillations in certain areas, created a system with dynamical properties normally not associated with this type of system. Another distinct feature of the cell arrays is the absence of bulk oscillations, i.e., in-phase synchronization across the entire system, for any initial condition. We argued that the characteristics of a single cell were comparable to those of an oscillatory yeast cell, and hence we predict that it could be difficult to observe bulk oscillations in a layer of oscillatory yeast cells fixed inside a gel [15].

Antispirals and antipacemakers have previously been observed in the BZ reaction taking place in a water-in-oil microemulsion [16,17] along with numerous other patterns, including Turing patterns [18]. In this case the water droplets and the oil (octane) were separated by the surfactant sodium bis(2-ethylhexyl)-sulfosuccinate (known as Aerosol OT or AOT). The dimension of the AOT system is on the nanoscale (much smaller than the wavelength of waves propagating through the system) regarding the water-phase droplets where the BZ reaction takes place, but there are also similarities. In both cases there are active parts separated by inactive ''transportation'' parts. This similarity along with the presence of Turing patterns in the BZ-AOT system raises the question of whether the development of stationary structures might be facilitated by the presence of oscillatory cells, as opposed to just oscillatory homogeneous media.

[1] C. Furusawa and K. Kaneko, Artif. Life **4**, 79 (1998).
[2] Y. Kuramoto, Int. J. Bifurcation Chaos Appl. Sci. Eng. **7**, 789 (1997).
[3] S. Schuster, M. Marhl, and T. Höfer, Eur. J. Biochem. **269**, 1333 (2002).
[4] H.R. Petty, R.G. Worth, and A.L. Kindzelskii, Phys. Rev. Lett. **84**, 2754 (2000).
[5] P. Richard, B.M. Bakker, B. Teusink, K. Van Dam, and H.V. Westerhoff, Eur. J. Biochem. **235**, 238 (1996).
[6] S. Danø, P.G. Sørensen, and F. Hynne, Nature (London) **402**, 320 (1999).
[7] J.J. Tyson and P.C. Fife, J. Chem. Phys. **73**, 2224 (1980).
[8] M. Hildebrand, H. Skødt, and K. Showalter, Phys. Rev. Lett. **87**, 088303 (2001).
[9] L. Kuhnert, Nature (London) **319**, 393 (1986).
[10] H.-J. Krug, L. Pohlmann, and L. Kuhnert, J. Phys. Chem. **94**, 4862 (1990).
[11] A.K. Ghosh, B. Chance, and E.K. Pye, Arch. Biochem. Biophys. **145**, 319 (1971).
[12] O. Steinbock, V.S. Zykov, and S.C. Müller, Nature (London) **366**, 322 (1993).
[13] S. Grill, V.S. Zykov, and S.C. Müller, Phys. Rev. Lett. **75**, 3368 (1995).
[14] S.C. Müller and T. Plesser, in *Chemical Waves and Patterns*, edited by R. Kapral and K. Showalter (Kluwer Academic, Dordrecht, 1995), pp. 57–92.
[15] S. Chia, J. Urano, F. Tamanoi, B. Dunn, and J.I. Zink, J. Am. Chem. Soc. **122**, 6488 (2000).
[16] V.K. Vanag and I.R. Epstein, Phys. Rev. Lett. **87**, 228301 (2001).
[17] V.K. Vanag and I.R. Epstein, Science **294**, 835 (2001).
[18] A.M. Turing, Philos. Trans. R. Soc. London, Ser. B **237**, 37 (1952).
[19] A detailed description of the setup can be found at http://theochem.ki.ku.dk/~cats/library/setup.pdf