Use of a Multioscillator System to Simulate Experimental Results Obtained for the *period-mutants* of *Drosophila melanogaster*

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Abstract

We propose a multioscillator model which includes as a novelty the presence of neutral elements, the physiological correlate of which may be glial cells. The neutral elements do not exhibit selfsustained oscillations but follow and influence the circadian fluctuations of the oscillators in their neighbourhood. We are able to simulate the different circadian behaviours of the *period*-mutants and the wildtype of *Drosophila melanogaster* by changing the number or weight of the neutral elements that interfere with the oscillators.

Introduction

In *Drosophila melanogaster* the *period* (*per*)-protein is of fundamental importance for circadian rhythmicity (Hall and Kyriacou, 1990). Mutations in the *per*-gene either shorten or lenghten the period of locomotor activity, or cause arrhythmicity. Immunocytochemical studies have shown that the *per*-protein is located in a large number of glial cells, in photoreceptor cells and in a few neurons, that were called "lateral neurons" (LNs) according to their position at the lateral margin of the brain (Ewer et al. 1992). The LNs are also specifically stained by an antiserum against a hormone, the pigment-dispersing hormone (PDH) (Helfrich-Förster 1993, Helfrich-Förster and Homberg, 1993). ThePDH-immunostaining revealed the arborization pattern of the LNs, and showed that the arborizations of the LNs are in close vicinity to *per* containing glial cells. Both cell types seem to have contact with each other.

The role of the *per*-protein containing glial cells in the circadian system of Drosophila is still unknown. Glial cells are assumed to lack selfsustained oscillations that contribute to the overt rhythm. However, circadian variations in the amount of *per*-protein are measurable in glial cells (Zerr et al., 1990). In the mammalian SCN glial cells follow rhythmic fluctuations which are measurable in neurons. On the other hand, they influence these fluctuations by acting as a kind of buffering capacity which stabilizes the electrolytic environment of neurons. Glial cells have been omitted from models of cellular interactions underlying circadian rhythms. However, as *per*-protein containing glia makes up the majority of *per*-containing cells, their possible participation in cellular interactions underlying circadian rhythms merits some attention.

We now propose a model which as a novelty introduces "neutral elements" , the physiological correlate of which may be glial cells. The neutral elements do not exhibit selfsustained oscillations, but follow and affect the fluctuations in their neighbourhood.

We assume that the *per*-protein affects the buffering capacity of the *per*-containing glial cells. Their buffering capacity may be high in per^s (*due* to a hyperactive protein), intermediate in the wildtype and low in per^l. In order to simulate the behaviour of per^s and per^l we changed the number and weight of the neutral elements (high number and weight in per^s, low number and weight in per^l).

Methods

Basic description of the model (Diéz-Noguera 1993)

Our system is based on a relatively high number of oscillatory units (more than 10) which are interconnected (coupled) through a network. The activity of the network can be modulated by external (light) or internal (feedback) factors. Each oscillatory unit is mathematically defimed by the Selkov equations (Selkov 1968), and its state is represented by a point in an x,y plane. The whole system of differential equations is numerically solved by a second order Runge-Kutta method.

The interactions (couplings) between the oscillatory units are defined by a linear function proportional to the physical distances among the elements and proportional to their weights. Light does not act directly on the oscillators but on the coupling between the elements. In darkness the coupling between the elements is high. With increasing light intensity the coupling between the elements is reduced. Entrainment to a light-dark cycle is achieved by cyclically changing the coupling strength.

Additionally to the oscillators the model includes neutral elements, defined mathematically by the same equations as the oscillators but with zero velocity. In the x,y plane they are points that follow the movements in their neighbourhood, and affect the state of their neighbours. Their influence on the oscillators depends on their number, their weight and on their physical distance to the oscillators.

Model adapted to the situation in Drosophila

Based on the bilateral distribution of the *per*-protein containing LNs, we used two groups of oscillators, both having the same distribution of frequencies. Since the number of the LNs in each group is about 7, we used 7 oscillatory units. We assumed that the coupling of the oscillators within each group is stronger than the coupling between oscillators of both groups. Therefore, we chose the distance between both groups larger than between the oscillators in the group. The neutral elements were positioned between the two oscillator groups. In order to simulate the behaviour of per^s and per^l we changed the number, weight or position of the neutral elements.

Modelling the behaviour of the *per-mutants* under increasing light-intensity

Under conditions of continuous light (LL) the period of the wildtype and of the *per*-mutants is lengthened with increasing light intensities (Helfrich-Förster, 1990). When periods are plotted as a function of light intensity, the slope of the curves is

different for the three strains (see Fig. 3a). In addition to lengthening the period, light seems to destabilize the circadian rhythmicity. With increasing light intensity the flies of all three strains become more active, tend to exhibit complex activity patterns and finally become arrhythmic. Complex activity patterns were observed at about 5 lux for the wildtype and per^l, and already at about 2 lux for per^s.

In the model, we tried to simulate the behaviour of the wildtype and that of the *per*-mutants under increasing light intensities by decreasing the internal coupling of the system.

Results

To test the effect of neutral elements on the circadian output of the oscillators in the model, two approaches were tried. In the first approach the number and the weight of neutral elements were varied (Fig. 1). In the second approach the position and weight of 2 respectively 14 neutral elements were changed (Fig. 2). For each system the periods at different coupling strengths (= different light intensities) were determined and are shown in a diagram as a function of coupling.

General observations

In all systems tested the following features are in accordance with the experimental results: (1) The period is lengthened when coupling is decreased (at least in a limited range of coupling), (2) the amount of activity becomes higher with decreased coupling and (3) complex patterns and arrhythmicity were observed at low coupling strength.

Influence of the neutral elements

The higher the weight of the neutral elements the shorter is the period of the oscillator system (Fig. 1, 2). The larger the number of neutral elements the earlier complex rhythmicity occurs when coupling is decreased (Fig. 1, 8n, 14n; Fig. 2b). Furthermore, the weights of the neutral elements affect the slope of the curves in the period/coupling diagrams. When the weight of the neutral elements is high the curves become similar to the curve observed for per^s. This effect becomes more pronounced with increasing numbers of neutral elements (Fig. 1).

The position of the neutral elements relative to the oscillators has also some effect on the curves in the period/coupling diagram (Fig. 2). When the distance between oscillators and neutral elements is small (D1 in Fig. 1), the region of the curve at which period is lengthened with decreasing coupling is very small. This region becomes longer when the distance between oscillators and neutral elements is increased (D4 in Fig.2).

Simulating the behaviour of the *per-mutants* and the WT under increasing light intensity

The behaviour of the per^l-mutant can be most successfully simulated by using a

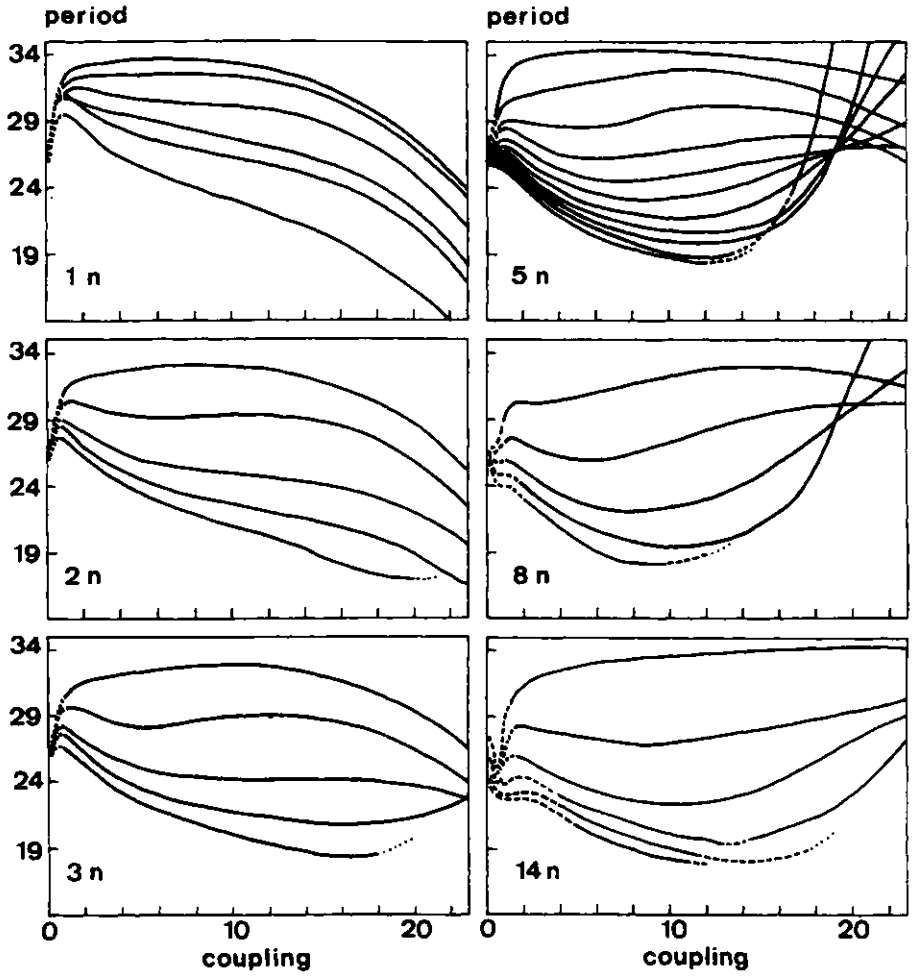

Fig. 1. Influence of number and weight of neutral elements on the period at different couplings. One (1n), 2 (2n), 3 (3n), 5 (5n), 8 (8n), and 14 (14n) neutral elements were present between the two groups of oscillators and stepwise increased in weight. In diagram "n1" the weights of the neutral elements were 0.2, 0.3, 0.5, 0.8, 1.0, 2.0, in diagram "n5" the weights were 0.05, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, and in all other diagrams they were 0.2, 0.5, 1.0, 1.5 and 2.0 respectively. In each diagram the upper curve represents the curve with the lowest weight of neutral elements. The broken lines in the diagrams indicate the occurrence of complex activity patterns. The distance between oscillators and neutral elements was the same (D = 40 mm) in all diagrams.

single neutral element (of low weight) situated in the middle of the two oscillator groups. The behaviour of the WT can be best simulated by using two neutral elements (of intermediate weight) situated in the middle of the two oscillator groups. The behaviour of the *per*^{s} mutant can be simulated by using several

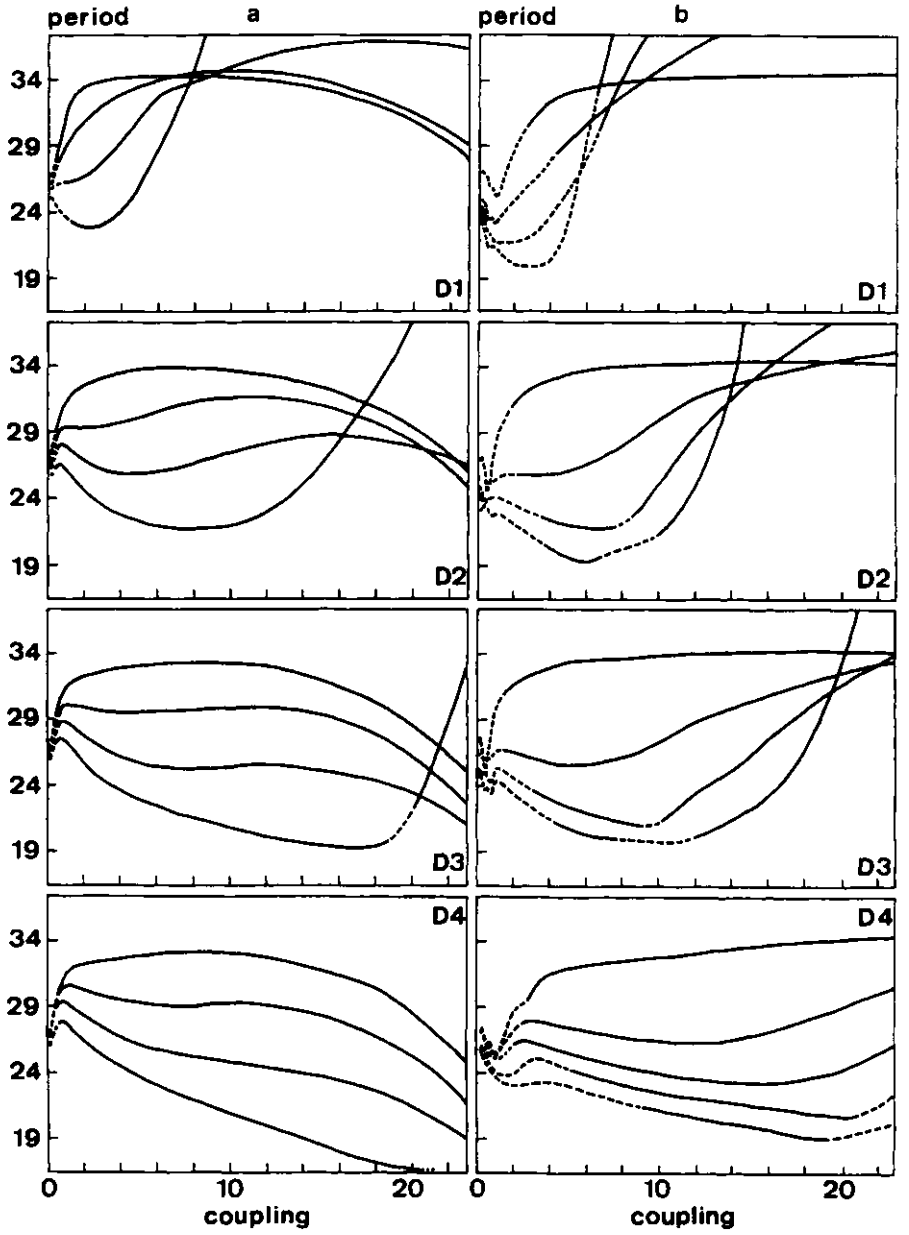

Fig. 2. Influence of the position of neutral elements on the period at different coupling strenghts. Two (a) and 14 (b) neutral elements were used. From D1 to D4 the distance between oscillators and neutral elements is stepwise increased. The distances between oscillators and neutral elements are as follows: D1 = 12, D2 = 24, D3 = 32, D4 = 48 (in mm, measured on the screen). The weights of the neutral elements were 0.2, 0.5, 1.0, and 2.0 respectively, the upper curve in each diagram representing the lowest weight.

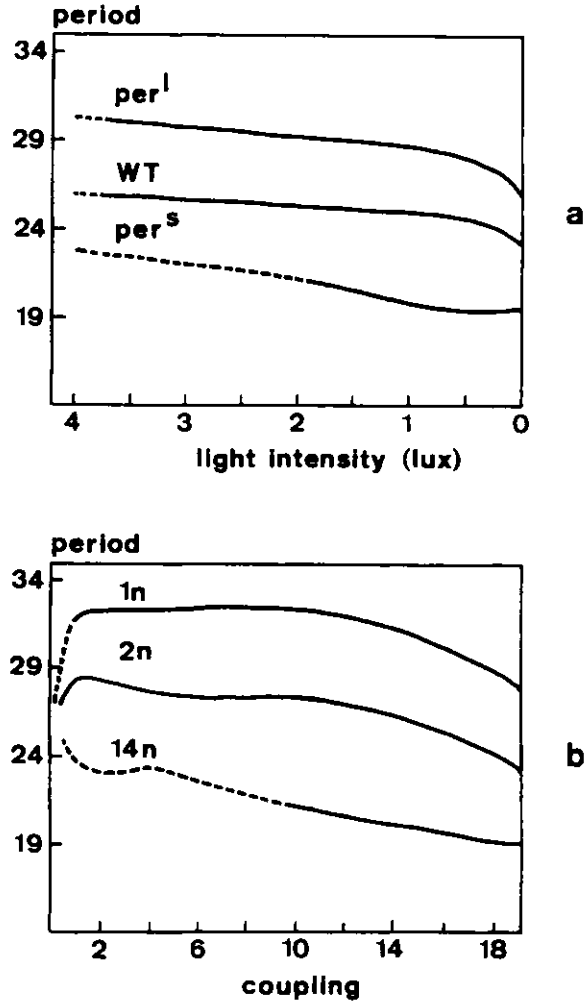

Fig. 3. (a) Periods of the wild-type Canton S (WT) and the mutants *per*^{s} and *per*^{l} as a function of light intensity. (b) Simulation of the period changes of the wild-type and the *per*-mutants under increasing light intensity (= decreasing coupling). For *per*^{l} one neutral element (weight: 0.2) was positioned between the two groups of oscillators, for the wildtype two (weights: 0.5) and for *per*^{s} 14 neutral elements (weights: 2.0) were used.

neutral elements (of high weight) situated in the middle between the two oscillator groups (best with 14 neutral elements, weight 2.0, D4 in Fig. 2). Fig. 3 compares diagrams of the simulated curves with the curves found experimentally.

Discussion

The results show that the presence of neutral elements has a strong influence on the output of the oscillator system. The overall period is shortened when the weight of the neutral elements is increased. This can be interpreted in physiological terms as a higher buffering capacity of the glial cells (might apply to *per*^{s}). The long period of *per*^{l} *is* achieved by assuming a small influence of neutral elements (low buffering capacity of the neutral elements). The behaviour of the wildtype may result from an intermediate buffering capacity of the neutral elements.

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Hypothesis: a Controlled Chaotic Attractor Constitutes the Central Oscillator of the Circadian Clock

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Control of chaos to give stable periodic behaviour has recently been demonstrated in physical (thermal conduction loop, magneto-elastic ribbon, Yttrium-iron-garnet oscillator, diode resonator, autonomously chaotic multimode laser)