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ARTICLE

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Ligand-receptor interaction under periodic stimulation: a modeling study of concentration chemoreceptors

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Abstract The first step of chemosensory transduction consists in the association of ligand molecules with receptor proteins borne by the cell membrane. In this article, the time evolution of ligand-receptor complexes is studied in the presence of a periodically changing ligand concentration. This type of stimulation is a close approximation to some natural situations, for example in olfaction. The transient and steady-state periodic levels of the complexes, resulting from a single-step (binding) or double-step (binding and activation) reaction, are determined. When possible, analytical solutions are given, if not for the complete model, at least for its simplified version at low ligand concentration. Otherwise, solutions are found numerically and both the complete and simplified versions of the model are compared. The results obtained are discussed with respect to actual experimental data based on the moth sex-pheromone receptor. Periodic steady states are achieved very quickly and their amplitude decreases when the stimulation frequency increases. We show that the simplified description is adequate if only a fraction of activated receptors is sufficient to produce the maximum response, as is actually the case in the example treated. The role of the frequency of stimulation is investigated and it is shown to possess an optimal range between 2 and 5 Hz.

Keywords Intensity coding · Olfactory receptor neuron · Perireceptor space · Insect sensillum · Frequency-dependent process

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Introduction

Most, if not all, cells are able to transduce chemical stimuli present in their environment into a chemical or electrical response. The ligand molecules can bind with receptor proteins located in the cell membrane which triggers specific responses (reviewed in Lauffenburger and Linderman 1993). Depending on ligands and cells, a single channel may be opened (e.g., nicotinic acetylcholine receptor) or an enzymatic active site be triggered (typically phosphorylating proteins on tyrosine residues) or a GTP-binding G-protein be activated. In the last case, the G-proteins may act in turn on enzymes to induce the generation of second messengers (e.g., cyclic AMP). In neurons and especially olfactory receptor neurons, which are at the center of our interest, these second messengers open a large number of ion channels, resulting in a change of the membrane potential and a subsequent firing of action potentials (see e.g., Torre et al. 1995).

Several models of ligand-receptor interactions have been already studied in the context of chemoreception by Beidler (1962), Ennis (1991), Getz (1999), Getz and Akers (1995), Kaissling (1987, 1998a, 1998b), Malaka et al. (1995), Lánský and Rospars (1993, 1995), and Rospars et al. (1996). They can be characterized as follows. First, they generally assume that the cell chemosensory membrane is directly exposed to the external environment, i.e. no physically distinct perireceptor space is present. Such systems were recently called *concentration detectors* by Kaissling (1998a) and distinguished from *flux detectors* which encompass a physically distinct perireceptor space (Rospars et al. 2000a). Second, two basic types of interaction of ligand molecules with the receptor proteins have been studied. In the first one, the transduction cascade is triggered by mere binding of the ligand to the receptor to form a complex (single-step interaction); in the second, additionally, an activation of the receptor-ligand complex is required (two-step interaction). Third, mostly the steady-state

responses were analyzed in the presence of a constant concentration of a ligand but rarely the transient responses resulting from a step or square stimulation.

This last feature, concerning the time independency of the stimulation, seems to be the most urgent to reconsider, while keeping the two other features. Concerning stimulation, the constant and step or square deliveries are mainly used in experimental conditions, while, in natural conditions, it has been shown that turbulence of the carrier medium, air or water, physically breaks the initially continuous ligand plume into spatially and temporally discontinuous patches (Dittmer et al. 1995; Kramer 1986; Moore and Atema 1991; Murlis 1997; Murlis and Jones 1981; Murlis et al. 1992). For a flying insect, such a spatially discrete plume is experienced as a discrete temporal signal. Similarly, in vertebrates, breathing also periodically changes the odorant concentration in the nasal cavity. Therefore, the aim of the present paper is to investigate the response of the one- and two-step systems when they are exposed to a periodically varying ligand concentration. Such a periodic stimulus is again an idealization, but it is a better approximation of natural odorant stimuli than the constant ones. Although we restrict ourselves to olfaction as an example, the present approach offers a much wider applicability, as many biological responses are dependent on the frequency of excitation. The models presented can describe not only the periodic switching of ligand concentration between two values but also the periodic change in conformation of the receptor, so that binding occurs only when the receptor transiently resides in a bindable state. Examples of frequency-dependent processes are synaptic depression, the blockade of ion channels by local anesthetics or by serotonin in the case of sensitization (see Starmer 1987, 1992).

As far as the concentration and flux detectors are concerned, we restrict our attention to the concentration detectors. However, if time fluctuation of the concentration in the vicinity of the membrane closely follows time fluctuation of the concentration in the external space, then the only effect of introducing the peri-receptor space is a substantial amplification of the internal concentration with respect to the environment. In this situation the flux detectors can be satisfactorily approximated by concentration detectors.

Theory

Signaling complexes

A patch of sensory membrane uniformly covered with identical receptors R of concentration N is considered. Ligand molecules can bind to receptors R and create various complexes. Let us denote by $R(t)$ the concentrations of free (“not interacting”) receptors and by $\bar{R}(t)$ the concentration of bound (“interacting”) receptors, so that $N = R(t) + \bar{R}(t)$. We assume that, up to time origin, no ligand is present at the vicinity of the receptors, which means that the initial conditions are $R(0) = N$ and $\bar{R}(0) = 0$. The number of different forms creating $\bar{R}$ depends on the complexity of the model. Only the cases with one and two forms are studied. In the first case, called *single-step interaction*, the cell response is triggered by the mere binding of the ligand to the receptor, forming a ligand-receptor complex denoted by C with concentration C ($\bar{R} = C$). In the second case, called double-step interaction, binding of the ligand is not sufficient to trigger the response; the bound complex must go through an additional step, which can correspond to allosteric or covalent modification, to produce an activated complex C* with concentration C^* ($\bar{R} = C + C^*$). The concentrations of C in single-step models and C* in double-step ones are the main variables studied in this paper. Both are referred to as the *signaling complexes*. More complex models with interconversion of receptor forms possessing different binding properties have not been, to our knowledge, used in a chemosensory context, and their introduction is beyond the scope of this paper. Further generalizations containing more than two steps, although formally complicated, can be achieved by analogy.

Sine-wave stimulation

The ligand molecules L are uniformly diluted in the carrier medium (water or air) which is in direct contact with the receptors. Two types of time dependency of the concentration of the ligand, $L(t)$, are studied. In the first one, at time t, the concentration is described by a sine wave:

$$L(t) = \begin{cases} L_0 + L_1 \sin(\omega t + \phi) & \text{for } t \geq 0 \\ 0 & \text{for } t < 0 \end{cases} \quad (1)$$

where the parameters are the amplitude L_1, angular frequency $\omega = 2\pi f$ (expressed in rad s^{-1}; f is frequency in Hz), and phase shift ϕ of the oscillatory component of the stimulation. The amplitude L_1 must be smaller than or equal to the level of the constant component L_0, so that $L(t) \geq 0$ is ensured. The main variable of interest in the periodic stimulation is its frequency, which may correspond to some external conditions, e.g., breathing in vertebrate animals or segmentation of the air plume in insects. On the other hand, the phase shift ϕ plays only a marginal and formal role in this study, so we set $\phi = 0$. Stimulation (1) is also interesting at its onset (small t) for studying the transient effects and mimicking a ramp-like stimulation. For large t, the effect of stimulus initiation disappears and we may wonder whether a periodic steady state can be achieved.

Pulsed stimulation

Stimulation (1) is only an abstraction of real processes which can arise under experimental as well as natural conditions. Its main disadvantage is that the lengths of “low” and “high” concentration intervals are the same. To take into account the situation where these intervals are different, we also investigate a second possible type of stimulation composed of alternating square pulses, in the form:

$$L(t) = \begin{cases} L_2 & \text{for } t \in [n(t_L + t_H),\ n(t_L + t_H) + t_H] \\ 0 & \text{for elsewhere} \end{cases} \quad (2)$$

where $n = \{0, 1, ...\}$, t_H is the duration of ligand application, and t_L is the duration of ligand absence (generalization for two levels L_{21} and L_{22} is straightforward). The stimulation frequency is $f = (t_L + t_H)^{-1}$. Apparently, the stimulation frequency in model (2) can be changed either by modifying t_L or t_H. In order to conform to actual experimental practice, we assume that L_2 and t_H are fixed in Eq. (2), and that t_L is variable. Of course, this assumption implies that the amount of ligand delivered per unit of time is different for different stimulation frequencies.

For comparing with the sine-wave stimulation, the relationship between L_0 and L_1 in Eq. (1) and L_2 in Eq. (2) must be specified. For example, $L_2 = L_0 + L_1$ ensures that for low frequency of stimulation ω and sufficiently long t_H, the maximum number of activated receptors is the same with both kinds of stimulations. On the

other hand, the condition $L_2 = L_0$ would mean that for sufficiently long t_H, the maxima of the signaling complex for pulses coincides with the mean value of the signaling complex achieved with sine waves. The second-order discontinuity of Eq. (2) at the points of concentration change is the opposite extreme to the smoothly changing level in Eq. (1). While the disadvantage of stimulation (1) is symmetry of its crests and troughs, the price to pay for removing it is the replacement of the single parameter ω by the couple t_L and t_H.

Analytical results

Single-step interaction

Time rate of change of the signaling complex C

In the simplest model, the transduction cascade is triggered by mere binding, $L + R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C$, where C denotes the complex RL, and k_1 and k_{-1} are the binding and release rate constants, respectively. Taking into account that $R(t) + C(t) = N$ is constant, only one independent equation can be written for the system:

$$\frac{dC(t)}{dt} = -(k_{-1} + k_1 L(t))C(t) + k_1 L(t)N \tag{3}$$

Equation (3) takes into account the limited number of receptor sites and implicitly assumes that the concentration $L(t)$ is not influenced by the binding and release of ligand molecules. In particular, it does not describe the detailed geometric properties of the membrane and related phenomena (Lagerholm and Thompson 1998).

If $k_{-1} >> k_1 L(t)$, which is equivalent to the condition that the number of bound receptors is always far below their total number, $C(t) << N$, Eq. (3) can be simplified into the form:

$$\frac{dM(t)}{dt} = -k_{-1}M(t) + k_1 L(t)N \tag{4}$$

where the concentration of the complexes is denoted by M. This equation is suitable whenever a low concentration of ligand is applied. The difference between M and C is illustrated below (see Numerical results) and has its counterpart in modeling electrical properties of the neuronal membrane (see Appendix A).

Sine-wave stimulation

The solution of Eq. (4) in the case of stimulation (1) is well known:

$$M(t) = \frac{k_1 N}{k_1}\left(L_0\left(1 - e^{-k_{-1}t}\right) + \frac{L_1 k_{-1}}{k_{-1}^2 + \omega^2}\left(k_{-1}\sin\omega t - \omega\cos\omega t - k_{-1}e^{-k_{-1}t}\right)\right) \tag{5}$$

The asymptotic form of solution (5) is achieved with time constant k_{-1} and it can be written in the form:

$$M_\infty(t) = k_1 N\left(\frac{L_0}{k_{-1}} + \frac{L_1 \sin\left(\omega t - \operatorname{arctg}\frac{\omega}{k_{-1}}\right)}{\sqrt{k_{-1}^2 + \omega^2}}\right) \tag{6}$$

The response of the signaling complex is periodic around the mean level NL_0k_1/k_{-1} and with the same period $2\pi/\omega$ as the stimulation. Its amplitude is:

$$A = \frac{k_1 N L_1}{\sqrt{k_{-1}^2 + \omega^2}} \tag{7}$$

from which it follows that the relative amplitude, A_r, with respect to the largest obtainable amplitude when $\omega \to 0$, is $A_r = k_{-1}/\sqrt{k_{-1}^2 + \omega^2}$. Amplitude A does not depend on L_0, which is implicitly assumed to be small. With increasing frequency of stimulation, A approaches zero proportionally to $1/\omega$. For large values of ω the asymptotic levels of constant (corresponding to $L_1 = 0$) and periodic stimulations coincide, since in this case the concentration of bound receptors oscillates very fast with a very small amplitude around $k_1 N L_0/k_{-1}$. The phase shift between M and stimulation can also be deduced from solution (6). The response is delayed after the stimulation by:

$$D_1 = \frac{1}{\omega}\operatorname{arctg}\frac{\omega}{k_{-1}} \tag{8}$$

so that the relative shift with respect to the period of stimulation is $D_1/T = \operatorname{arctg}\left(\frac{\omega}{k_{-1}}\right)\Big/2\pi$. Analyzing D_1/T as a function of ω, it can be seen that at low frequency of stimulation the relative delay of the response is negligible. With increasing stimulation frequency ω, the response is more and more delayed after the stimulation, up to one quarter of the period of stimulation. The role of the dissociation rate k_{-1} in Eqs. (7) and (8) is opposite to the role of ω.

The effect of the periodicity of the stimulation can be best appreciated by determining the stimulation which yields in some sense the optimal response of the system. In agreement with observations in several insect species, this stimulation must meet two conditions. First, the amplitude of the periodic change in the concentration of the signaling complex must be sufficiently large. Second, the stimulation must maximize the change of the complex per unit of time. So, the product of the amplitude by the change per unit of time appears as a suitable candidate to determine the optimal response. This can be best illustrated on the simplified single-step model. The amplitude A is given by Eq. (7) and, taking the derivative of Eq. (6) with respect to time, the maximum speed at which $M_\infty(t)$ changes is $(\omega k_1 N L_1)/\sqrt{k_{-1}^2 + \omega^2} = \omega A$. Thus, the suggested measure of the tuning γ caused by periodic stimulation is $A^2\omega$, which takes for model (4) the form:

$$\gamma(\omega) = \frac{(k_1 N L_1)^2 \omega}{k_{-1}^2 + \omega^2} \tag{9}$$

This function tends to zero for high as well as low stimulation frequencies and reaches its maximum at $\omega_m = k_{-1}$. So, the optimum frequency of the stimulus is $f_m = k_{-1}/2\pi$.

Solution of the complete model (3) for sine-wave stimulation does not exist in analytical form but its asymptotic periodicity is proved in Appendix B. In this case the tuning curve γ and its maximum can be calculated numerically (see Numerical results below).

Pulsed stimulation

Contrary to the sine-wave stimulation with stimulation (2), the solution for the complete model is available. The concentration of the signaling complex reaches a periodic steady state in which each pulse gives rise to a distorted response with a saw-like appearance. The asymptotic maximum level of bound receptors (peak of saw teeth) is:

$$C_{max} = \frac{k_1 N L_2 (1 - \exp(-(k_{-1} + k_1 L_2) t_H))}{(k_{-1} + k_1 L_2)(1 - \exp(-(k_{-1} t_L + (k_{-1} + k_1 L_2) t_H)))} \tag{10}$$

and for the minimum we have:

$$C_{min} = C_{max} \exp(-k_{-1} t_L) \tag{11}$$

Between these two extremes, the function $C(t)$ alternatively grows exponentially (with time constant $k_{-1} + k_1 L_2$) to the asymptotic level $k_1 N L_2/(k_{-1} + k_1 L_2)$ and decays (with time constant k_{-1}) to zero. From extremes (10) and (11) the amplitude A can be defined as half of the difference C_{max}–C_{min}:

$$A = \frac{k_1 N L_2 \left(1 - e^{-(k_{-1} + k_1 L_2) t_H}\right)}{2(k_{-1} + k_1 L_2)} \frac{1 - e^{-k_{-1} t_L}}{1 - e^{-(k_{-1} t_L + (k_{-1} + k_1 L_2) t_H)}} \tag{12}$$

which tends to zero for $t_L \to 0$ (high-frequency stimulation). Another limiting case of interest is that for which the intervals of ligand application are very short, the concentration of ligand is very high, formally $t_H \to 0$ and $L_2 \to \infty$, and the product of these two quantities is constant or at least asymptotically constant (the amount of ligand delivered per pulse does not change), $t_H L_2 = q$. Then Eq. (10) takes the form:

$$C_{max} = \frac{N(1 - \exp(-k_1 q))}{1 - \exp(-(k_{-1} t_L + k_1 q))} \tag{13}$$

and the amplitude can be written directly:

$$A = \frac{N}{2} \frac{1 - \exp(-k_1 q)}{1 - \exp(-(k_{-1} t_L + k_1 q))} (1 - \exp(-(k_{-1} t_L))) \tag{14}$$

Combined with long periods without ligand or a fast deactivation rate, amplitude (14) yields:

$$A = \frac{N}{2}(1 - \exp(-k_1 q)) \tag{15}$$

Owing to the asymmetry of the stimulation, the term amplitude does not have the same obvious interpretation as in the case of the sinusoidal stimulation. Nevertheless, similarly to tuning of Eq. (9), we can search for an optimum silent interval t_L using the same concept, which leads us here to choose $\gamma(t_L) = A^2/t_L$ as the tuning function. Equation (12) for A permits only a numerical determination of the maximum of function γ. For illustrative purposes, let us assume that L_2 is sufficiently large and thus C_{max} almost reaches $k_1 N L_2/(k_{-1} + k_1 L_2)$. Then:

$$\gamma(t_L) = \left(\frac{1}{2} \frac{k_1 N L_2}{k_{-1} + k_1 L_2}\right)^2 \frac{(1 - \exp(-k_{-1} t_L))^2}{t_L} \tag{16}$$

with an approximate solution $t_L = 1/(2k_{-1})$, which corresponds very well to the value obtained for stimulation (1) from Eq. (9) (if $t_H = t_L$, the frequency is again k_{-1}).

Double-step interaction

*Time rate of change of the signaling complex C**

Adding a further step in the interaction scheme, of course, makes the model more realistic but simultaneously less tractable. Therefore, analytical results analogous to those presented in the previous section are lacking, or, even if the results can be derived, they are notationally so complex that they become of questionable interest. For this reason we focus mostly on qualitative consequences brought about by the addition of another step.

The reaction scheme $L + R \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C^*$ is a generalization of the previous model, where k_2 and k_{-2} are rate constants characterizing the velocities of the activation (C→C*) and deactivation (C*→C), respectively. It assumes that the receptors may appear in three states, unoccupied R, occupied and not activated C, occupied and activated C*, with $R = C + C^*$. For the concentration C of occupied receptors, and the concentration C^* of activated receptors, two equations can be formulated, taking again into account that at any time $R(t) + C(t) + C^*(t) = N$ is constant. We have:

$$\frac{dC(t)}{dt} = -(k_{-1} + k_1 L(t) + k_2) C(t) + (k_{-2} - k_1 L(t)) C^*(t) + k_1 L(t) N \tag{17}$$

and:

$$\frac{dC^*(t)}{dt} = -k_{-2} C^*(t) + k_2 C(t) \tag{18}$$

If the same low-concentration approximation as that described in the previous section, which lead to Eq. (4), is used, the description of the system can again be

substantially simplified. Now assuming that $k_{-1} \gg k_1 L(t)$ and $k_{-2} \gg k_1 L(t)$, instead of Eq. (17) we obtain:

$$\frac{dM(t)}{dt} = -(k_{-1} + k_2)M(t) + k_{-2}M^*(t) + k_1 L(t)N \qquad (19)$$

whereas Eq. (18) remains unchanged (only with different notation):

$$\frac{dM^*(t)}{dt} = -k_{-2}M^*(t) + k_2 M(t) \qquad (20)$$

Again, the stimulation appears in Eq. (19) as an additive term only.

Sine-wave stimulation

An analytical solution for $M^*(t)$, under stimulation (1), can be obtained, but it is notationally complicated. We only present the asymptotic formula for the periodic steady state $M^*_\infty(t)$:

$$M^*_\infty(t) = \frac{k_1 k_2 L_0 N}{k_{-1} k_{-2}} + \frac{k_1 k_2 L_1 N}{A_2 - A_1} \times \left(\frac{\omega \cos \omega t + A_1 \sin \omega t}{A_1^2 + \omega^2} - \frac{\omega \cos \omega t + A_2 \sin \omega t}{A_2^2 + \omega^2} \right) \qquad (21)$$

where $A_1 = -(k_{-1} + k_2 + k_{-2} + A_0)/2$, $A_2 = -(k_{-1} + k_2 + k_{-2} - A_0)/2$, and $A_0^2 = k_{-1}^2 + 2k_{-1}(k_2 - k_{-2}) + (k_2 + k_{-2})^2$. After some calculation, we can find the amplitude:

$$A = \frac{k_1 k_2 L_1 N}{(A_1^2 + \omega^2) left (A_2^2 + \omega^2)} \sqrt{\omega^2 (A_2 - A_1)^2 + (A_2 A_1 - \omega)^2} \qquad (22)$$

and the delay:

$$D_2 = \frac{1}{\omega} \mathrm{arctg} \frac{(A_2 + A_1)\omega}{A_2 A_1 - \omega} \qquad (23)$$

It can be seen in Eq. (22) that the trend to zero amplitude with increasing frequency is much faster here than in the one-step model (proportionally to ω^{-3}). It seems hardly possible to calculate the optimum frequency directly from Eq. (22), contrary to the single-step model.

Pulsed stimulation

A result analogous to Eq. (12) can be derived for the two-step model under periodic stimulation (2). However, it is again notationally hardly tractable. Also, the optimum length of the silent intervals t_L can be found only numerically. Further, a faster trend to lower amplitudes for decreasing t_L than in the single-step model could be proved. Thus, we can expect that longer intervals of silence are needed to achieve the same amplitude as in the single-step model.

Numerical results

Parameters and variables

The aim of this section is not to present a detailed quantitative characterization of a specific chemoreceptor under specific stimulation conditions, but to illustrate the influence of the periodic stimulation on the general behavior of the system. The total concentration of receptors N and the rate constant k's are intrinsic parameters of the system which are considered as fixed, while L_0, L_1, L_2, ω, t_H, and t_L are external parameters which can be modified. For this reason we investigate only the effect of these extrinsic parameters on the shape of the response. The main variables of interest are the frequency f in the case of the sine-wave stimulation and the durations t_H and t_L of on and off intervals in the case of the pulsed stimulation.

In our recent paper (Rospars et al. 2000a) on comparison of concentration detectors with other types of chemoreceptors, the numerical values of the parameters N, k_1, and k_{-1} characterizing the membrane were based on extensive experimental observations presented in Kaissling (1998a) for the sex-pheromone receptor neuron of the male moth *Antheraea polyphemus*. The same parameters are used here: $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}, $k_2 = 20$ s^{-1}, $k_{-2} = 5$ s^{-1}, $N = 10$ μmol L^{-1} (the concentration of the receptor proteins is expressed with respect to the volume of the hair lumen). In this species, as in other species investigated, the physiologically effective frequencies of stimulation f lie approximately in the interval 1–10 Hz (Rumbo and Kaissling 1989). This receptor neuron is not a mere concentration detector because a physically distinct perireceptor space is created by the multiporous hair cuticle that houses the sensory dendrite. In Rospars et al. (2000a) we showed that the main effect of interposing this perireceptor space was to increase the concentration L there with respect to the concentration L_{ex} in the environment. With the parameters chosen for describing the perireceptor space, L was found to be 10^6 times greater than L_{ex}. Consequently, with a constant level $L_{ex,0}$ of the stimulation lying in the interval 10^{-7}–10^{-3} μmol L^{-1}, as assumed here, the level L_0 in the perireceptor space is in the range 0.1–1000 μmol L^{-1}. The responses of the models can be expected to be gradual in this region.

In most examples we use an intermediate amplitude of the stimulus, $L_1 = L_0/2$. Note that for $L_1 = L_0$ the periodic component achieves its maximum possible effect, i.e., the stimulus concentration oscillates between 0 and $2L_0$. Thus, under the condition $L_2 = 2L_0$ and for $t_H = t_L$, both sine-wave and pulsed stimulations should behave in a similar way.

Comparison of complete and simplified descriptions

What is the difference between the full description of Eq. (3) and its approximation in Eq. (4)? We know that

this difference increases with the level of stimulation and consequently with the number of activated receptors. We also know that the amplitude of the concentration of the signaling complex, due to the periodic modulation, decreases with the stimulation frequency. For low stimulation frequency, the maxima of the number of activated receptors are identical with the steady states of the number of activated receptors stimulated by a constant stimulus $L = L_0 + L_1$. Thus, the agreement between the complete and the simplified descriptions increases with the stimulation frequency and tends to that achieved for the constant stimulus L_0. The maximum difference between both descriptions is illustrated in Fig. 1. We can see that the difference starts to be apparent at $L_0 = 10$ μmol L^{-1}, where it reaches 7% of the total number of receptors. At the highest frequency applied in this numerical investigation, $f = 10$ Hz, the difference decreases to 3.5% (Fig. 2). So, for approximately half of the range (in decadic scale) we are interested in, the approximation appears suitable. Any further increase of the stimulation intensity leads to a conspicuous difference between the two descriptions. The fact that the agreement is not substantially improved by increasing the frequency of stimulation comes from the relatively fast activation/deactivation rates chosen. On the other hand, Fig. 2 shows that curves $C(t)$ and $M(t)$ are rather well synchronized and of approximately the same amplitudes. Thus, Eqs. (7) and (8) for relative amplitude and relative delay between the response and the signal can be applied also for characterizing the complete description.

At first sight it could be expected that if $C(t)$ can be approximated by $M(t)$ then $C^*(t)$ can be also well approximated by $M^*(t)$, but this is not true. Our choice of parameters ensures that whereas up to $L_0 = 10$ μmol L^{-1} the single-step description can be simplified, the same does not hold for the double-step model. In this model the difference also depends on the condition $k_{-2} > > k_1 L$, which is not fulfilled at 10 μmol L^{-1}.

Concentration of signaling complex as a function of time

It can be seen in Fig. 2 that, for short t_H and longer t_L, $C(t)$ is below the concentration of the signaling complex under sinusoidal stimulation. The asymmetric shape of $C(t)$ in the case of pulsed stimulation (2) in comparison with sine wave (1) is apparent. The periodic steady state is definitely achieved after 0.4 s. This is, of course, faster than in the two-step model where binding and activation are employed (see Fig. 3), which needs 1.5 s for reaching the periodic steady state. Figure 3 also clearly illustrates a strong dependency of the response amplitude on the stimulation frequency. For the higher frequency of stimulation (2.5 Hz) the modulation of the number of activated receptors is apparently lower than for slowly varying signal (1 Hz); for 10 Hz (not shown) the amplitude is practically eliminated. The situation is analogous in the case of pulsed simulation. There also the amplitude is lower with decreasing silent periods t_L, but it is accompanied with an increase of the mean level around which the number of signaling complexes oscillates because the amount of ligand delivered per time unit increases. Finally, for ω very large and t_L very short the curves coincide asymptotically at the level 4 μmol L^{-1}.

The responses of single- and double-step systems are compared in Fig. 4. Owing to the selection of the rate constants, the baseline of the number of signaling

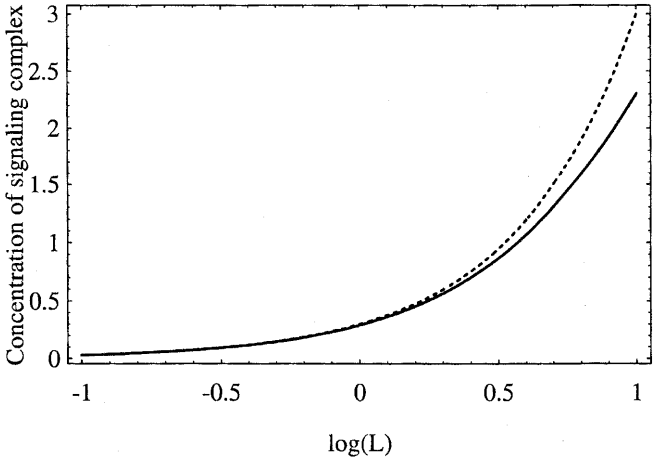

Fig. 1 Comparison of the concentrations of signaling complex in complete and simplified (low concentration) descriptions of single-step receptor-ligand interaction for constant stimulation $L = L_0 + L_1$. The concentrations (in μmol L^{-1}) C (complete model, *solid line*) and M (approximation, *dashed line*) are plotted against concentration L in decadic logarithmic scale, with $L_1 = L_0/2$. Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}

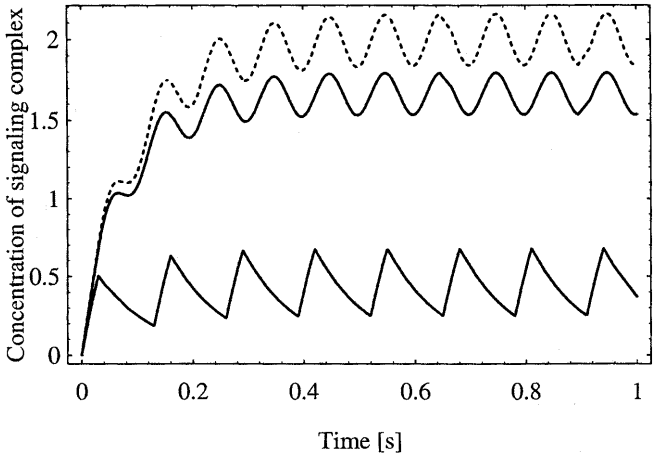

Fig. 2 Concentrations of signaling complex $C(t)$ (*solid lines*) and $M(t)$ (*dashed line*) for single-step interaction as a function of time for periodically changing stimulations. *Upper curves* with sine-wave stimulation (Eq. 1), *lower curve* with pulsed stimulation (Eq. 2). Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol $L^{-1}s^{-1}$, $k_{-1} = 10$ s^{-1}, $L_0 = 10$ μmol L^{-1}, $L_1 = 5$ μmol L^{-1}, $f = 10$ Hz; $L_2 = 10$ μmol L^{-1}, $t_H = 0.03$ s, $t_L = 0.1$ s

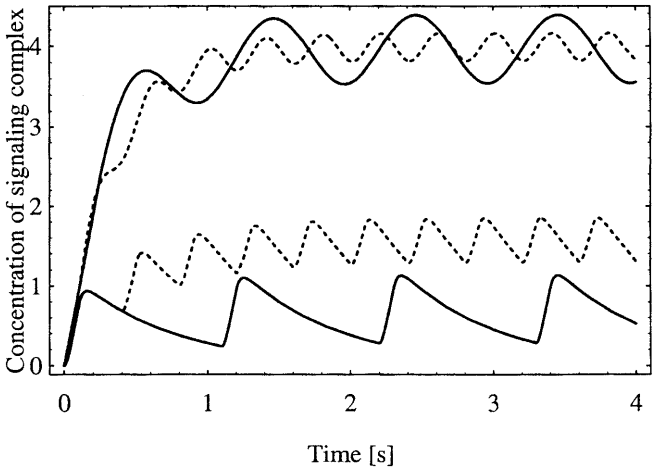

Fig. 3 Concentrations of signaling complex $C^*(t)$ for double-step reaction as a function of time for periodically changing stimulations at low frequency (*solid lines*) and high frequency (*dashed lines*). *Upper curves* with sine-wave stimulation (Eq. 1), *lower curves* with pulsed stimulation (Eq. 2). Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}, $k_2 = 20$ s^{-1}, $k_{-2} = 5$ s^{-1}, $L_0 = 10$ μmol L^{-1}, $L_1 = 5$ μmol L^{-1}, $L_2 = 10$ μmol L^{-1}, $t_H = 0.1$ s; *dashed lines* $f = 2.5$ Hz or $t_L = 0.3$ s and *full lines* $f = 1$ Hz or $t_L = 1$ s

receptors is higher in the double-step system $C^*(t)$ than in the single-step one $C(t)$. Thus, the approximation by simplified equations cannot be used (the error is about 100%). However, despite higher constant baseline, the amplitude is lower for $C^*(t)$; the amplitude for one-step interaction in Fig. 2 at stimulation frequency 10 Hz contrasts with the practically constant response at the same frequency for the two-step system in Fig. 3.

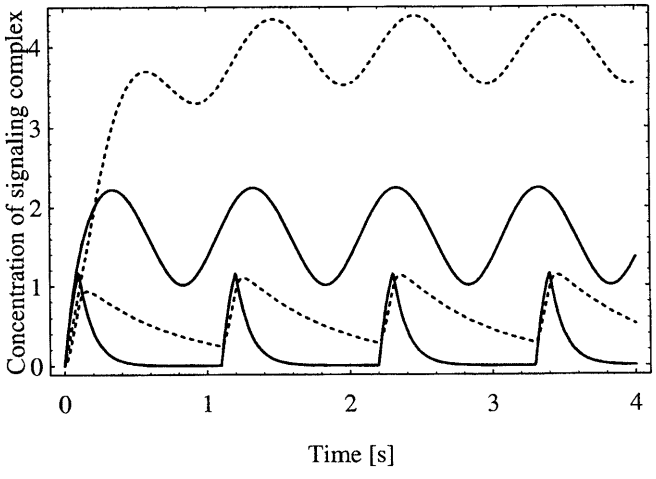

Fig. 4 Concentrations of signaling complex $C(t)$ for single-step reaction (*solid lines*) and $C^*(t)$ for double-step reaction (*dashed lines*) as a function of time for periodically changing stimulation. Sinusoidal responses (*top*) are evoked by sine-wave stimulation (Eq. 1), saw-like responses (*bottom*) by pulsed stimulation (Eq. 2). Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}, $k_2 = 20$ s^{-1}, $k_{-2} = 5$ s^{-1}, $L_0 = 10$ μmol L^{-1}, $L_1 = 5$ μmol L^{-1}, $L_2 = 10$ μmol L^{-1}, $f = 1$ Hz, $t_H = 0.1$ s, $t_L = 1$ s

Delay and tuning

The delay in response caused by the addition of a second step is apparent in Fig. 4. In Fig. 5 the delay of the response is shown in more detail, being based on the approximation in the single-step model. We can see that the absolute value of the delay decreases with the stimulation frequency, whereas the relative delay, with respect to the stimulation period, tends to 1/4. However, the large relative delay under the fast stimulation conditions does not play any important role because the amplitude of the response tends quickly to zero (see Numerical results below). Therefore, we may ask for which stimulation frequency the relative effect of the periodicity is largest.

For answering this question, the values of the tuning function γ given by Eq. (9) are plotted in Fig. 6 against the frequency together with analogous values calculated for both complete versions of the model, Eq. (3) (single-step) and Eqs. (17) and (18) (double-step). The results suggest that, at least for the parameters used in this example, the most efficient range of stimulation frequencies is around 2 Hz in the case of one-step model and even below 1 Hz for two-step model. The shift to the lower frequencies in the case of the more complex model is intuitive as there the decay of the amplitude with increasing frequency is faster. Figure 7 shows the tuning curves for the one-step reaction with pulsed stimulation (2). It shows that the optimum length of the silent interval t_L is close to 0.125 s, which means that the optimal stimulation frequency is 3 Hz with a duration of stimulation of $t_H = 0.2$ s. By decreasing the stimulation interval t_H and realizing that the length of the silent interval t_L is practically independent of it, much higher optimum frequencies are obtained, up to 10 Hz. In general, the values derived for the pulsed stimulation are larger than those derived for the sinusoidal stimulation.

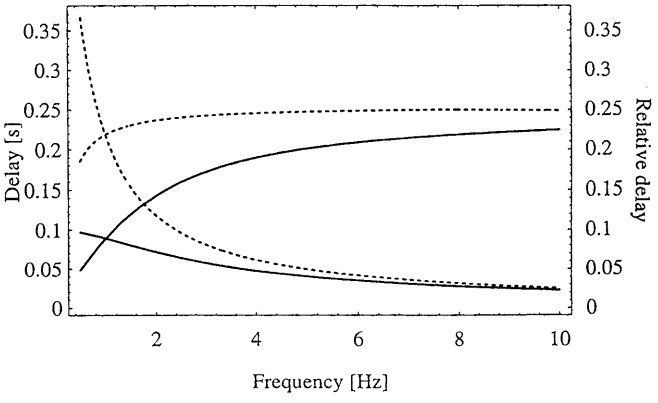

Fig. 5 Delay of response with respect to stimulation as a function of stimulation frequency f in the single-step reaction in the low concentration approximation $M(t)$ (*solid decreasing curve*) and $M^*(t)$ (*dashed decreasing curve*). Relative delay D with respect to the period of stimulation for $M(t)$ (*solid increasing curve*) and $M^*(t)$ (*dashed increasing curve*). Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}, $k_2 = 20$ s^{-1}, $k_{-2} = 5$ s^{-1}

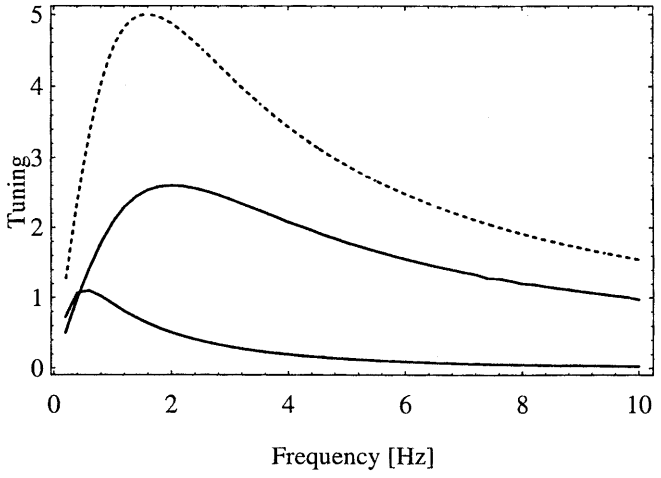

Fig. 6 Tuning curve γ with sine-wave stimulation (Eq. 1) for the single-step model (*middle solid line*), its low concentration approximation (*dashed line*), and for the double-step model (*lower solid line*) as a function of stimulation frequency f. Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}, $k_2 = 20$ s^{-1}, $k_{-2} = 5$ s^{-1}, $L_0 = 10$ μmol L^{-1}, $L_1 = 5$ μmol L^{-1}

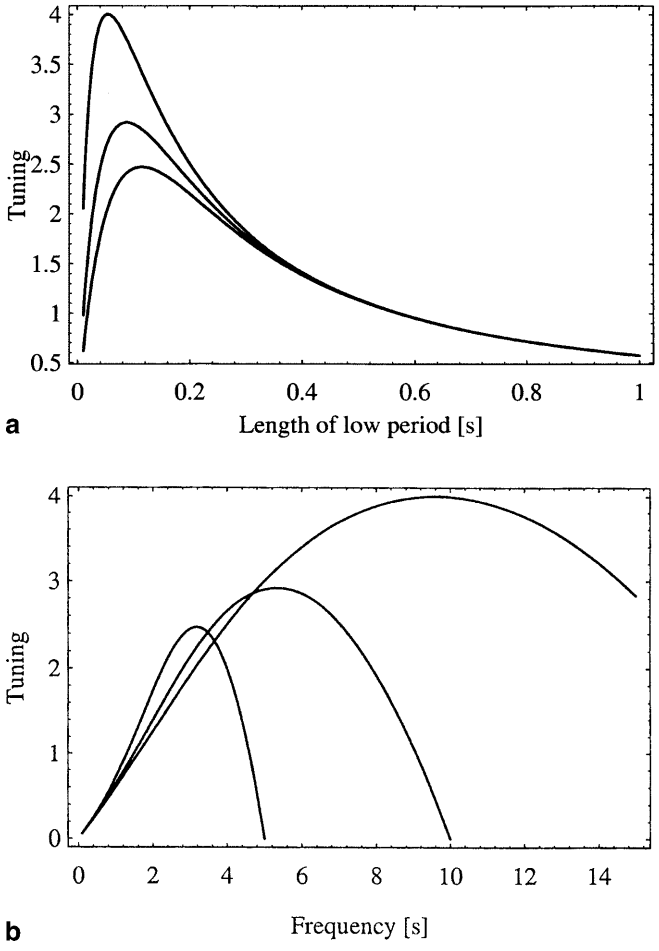

Fig. 7 Tuning curve γ with pulsed stimulation (Eq. 2) for single-step model as a function of **a** the length of the silent interval t_L (from *bottom to the top* $t_H = 0.2$, 0.1, and 0.05 s), and **b** the frequency $1/(t_L + t_H)$ (same values of t_H from *left to right*). Parameters: $N = 10$ μmol L^{-1}, $k_1 = 0.2$ μmol L^{-1} s^{-1}, $k_{-1} = 10$ s^{-1}, $L_2 = 10$ μmol L^{-1}

Discussion

Importance of concentration detectors

The concentration detectors are the simplest and most commonly used models for describing the early stages of information transfer in olfactory systems. Basically, they make no distinction between the concentration of an odorant in the external space L_{ex} and its concentration close to the sensory membrane. Their importance stems not only from their simplicity but also from their adaptability to more complicated types of detectors actually observed in nature.

In our previous paper (Rospars et al. 2000a) we investigated the response of concentration and flux detectors, i.e., systems without and with a distinct perireceptor space and ligand degradation, as originally defined by Kaissling (1998a). We also introduced a "generalized detector", in which the perireceptor space is characterized by two reaction rate constants, k_i describing the influx of ligand and k_{-i} its removal (outflux or degradation). In this case the equilibrium ratio between L and L_{ex} is equal to k_i/k_{-i} (which can be called the influx equilibrium constant K_i). Thus, a sufficiently fast influx with a relatively negligible outflux may ensure that the internal concentration is substantially higher than the external one. More exactly, if no other steps modify concentration L, as in our present models, then the ratio between the perireceptor concentration and the external one is equal to k_i/k_{-i}. An amplification occurs if this ratio is greater than one, with the consequence that the response curves of the generalized detector are shifted into the lower concentrations with respect to concentration detector with the same rate constants, as discussed in Rospars et al. (2000a) based on experimental measurements in the moth olfactory system (e.g., Kaissling 1998a; Zack 1979). It can be concluded that the results derived for concentration detectors are true even in the case when there exists a physically distinguishable perireceptor space, but the influx into it is so fast that the inner concentration tracks in time sufficiently closely the external one. This remark applies especially to the carbon dioxide detectors of insects, because unlike sex-pheromone molecules, carbon dioxide is only moderately lipophilic and is unlikely to be adsorbed irreversibly to the cuticle of a sensillum (Stange et al. 1995).

The description of the receptor-ligand interaction can be substantially simplified, if the number of signaling complexes remains small with respect to the total number of receptors. Description through the simplified equations seems to be interesting not only because of its tractability and versatility, but first of all because it seems to offer a satisfactory description of actual chemosensory systems. Indeed, various arguments

(Cleland and Linster 1999; Rospars et al. 2000a, 2000b) suggest that only a small fraction of the receptors has to be activated to obtain the maximum response. This description permits us to quantify the role of periodical stimulation and it is a question for further experimental verification if the measure proposed for finding an optimum stimulation frequency is the most suitable one. Note, however, that with our choice of parameters the low-concentration approximation works better with the single-step detector than with the double-step one. In the latter case the error with respect to $C^*(t)$ can reach 100%, whereas in the same concentration range $C(t)$ is well approximated by $M(t)$.

Role of the stimulation frequency

In what follows we call "response of the system" the concentration C or C^* of the signaling complex. The most obvious property of the response is that in all cases, triggered by a single- or a double-step reaction and stimulated by a sine wave or pulses, it achieves, after a certain delay, a periodic behavior which follows the stimulus with the same frequency. However, three characteristics of the response depend on the stimulation frequency: its amplitude, its time rate of change, and its delay with respect to the stimulus.

First, the amplitude of the response decreases with increasing frequency. For sufficiently slow frequency, the amplitude of the response approaches a maximum which is equal to the level that would be achieved by a constant stimulation $L = L_0 + L_1$ (sine) or $L = L_2$ (pulse). With increasing frequency, the response, although still periodic, does not reproduce the upper and lower parts of the signal, so that the amplitude of the oscillating part of the response decreases. Finally, the system averages out almost completely the quickly alternating signal, which results in a practically constant response. The level around which the response is modulated is the steady-state level observed under constant stimulation. This constant level can be also achieved by decreasing the amplitude of the stimulation.

Second, the time rate of change of the response increases with the frequency. In order to maximize both amplitude and rate of change simultaneously, we consider their product as a new measure of tuning between stimulus and response. This quantity reflects the importance for an insect, for example, of assessing simultaneously the amplitude of the stimulus and its time rate of change, both of which must not be too small since a constant stimulation is not perceived (Kennedy et al. 1980, 1981; Willis and Baker 1984). With this measure and the parameter values chosen, an optimum frequency is found ca. 2 Hz for sine waves and ca. 3–5 Hz for pulses.

Third, the delay of the response with respect to the stimulus decreases with the frequency but the relative delay with respect to the period of the stimulus increases.

This delay becomes important to know in the case when the time to reach a given threshold is investigated. It is apparent from the description of the model given by Eqs (3) or (17) that the concentration of the ligand appears always in a product with the binding rate constant k_1. Therefore, all results presented in this article are valid not only for periodically changing stimulus concentration but also for periodically fluctuating binding rate. For examples that lead to this scenario, see Starmer (1992).

Role of the number of receptor-ligand interaction steps

The mean level, amplitude, and reaction time of the response of double-step systems are different from those of their one-step counterparts. In the case where the deactivation equilibrium constant $K_2 = k_{-2}/k_2$ is smaller than one, as in our numerical example, the mean level of the double-step detector is always greater than that of the one-step, whatever the frequency of stimulation. This is not true for the amplitude, time to each equilibrium, and delay (see previous section), so we restrict the discussion to a relatively low stimulation frequency of one or a few hertz, close to the optimum stimulating frequency of the system for the parameter values chosen. Then, the double-step detector presents a slightly smaller amplitude and reacts more slowly, as can be seen by the time it needs to reach equilibrium (at onset of stimulation) and its greater smoothing of the sine waves and pulses (see Fig. 4). Therefore, the main advantage of introducing a second step with $K_2 < 1$ would be to increase the strength of the response (mean level) and consequently the sensitivity of the detector, since the same response strength is reached at lower concentration. However, the tradeoff for this increase in sensitivity is a lower contrast (amplitude) and time resolution, i.e., the ability to follow exactly the time variation of the ligand concentration is sacrificed to the ability to finely discriminate between the presence and absence of the ligand.

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Appendix A: comparison of chemical and neuronal models

Equation (3) describing the binding of ligand L to receptor R resembles formally the often-studied leaky-integrator neuronal model $dx/dt = -x/\tau + \mu(t)$ (Tuckwell 1988), in which x is the electrical potential of the neuron membrane, μ is the input (voltage change per unit time due to the stimulation), and τ is the time constant of the membrane. However, the two models differ because in

Eq. (3) the input force $k_1L(t)$ also enters the leakage constant $1/\tau$ of the neuronal model. Despite the models formally coinciding for a constant stimulation, that is when $L(t)$ and $\mu(t)$ are constant, the variability of this constant modifies the linear term in Eq. (3) whereas in the leaky integrator it remains unchanged. In fact, the model of binding considered here corresponds to the neuronal model with reversal potentials (Lánský and Lánská 1987), in which the voltage is restricted to the interval between inhibitory and excitatory reversal potentials. Similarly here, $C(t)$ is restricted to the interval $(0, N)$. Obviously, if Eq. (3) is replaced by Eq. (4) we obtain the classical leaky-integrator model.

Appendix B: proof of periodicity of dynamic response

Let us define:

$$P(t) = \exp\left(\frac{k_1 L_1 \cos \omega t}{\omega}\right) \tag{A1}$$

Using the transformation $C(t) = P(t)Z(t)$, Eq. (3) under the input signal (1) becomes:

$$\frac{dZ}{dt} = -(k_{-1} + k_1 L_0)Z(t) + \exp\left(-\frac{k_1 L_1 \cos \omega t}{\omega}\right) k_1 N L(t) \tag{A2}$$

Any solution of this equation has the following form:

$$Z(t) = \exp(-(k_{-1} + k_1 L_0)t)Z(0) + \int_0^t k_1 N L(\tau) \exp\left(-\frac{k_1 L_1 \cos \omega t}{\omega} - (k_{-1} + k_1 L_0)(t - \tau)\right) d\tau \tag{A3}$$

The periodic solution of the above equation is obtained if one sets:

$$Z(0) = \frac{\int_0^{\frac{2\pi}{\omega}} k_1 N L(\tau) \exp\left(-\frac{k_1 L_1 \cos \omega t}{\omega} + (k_{-1} + k_1 L_0)\tau\right) d\tau}{\exp\left((k_{-1} + k_1 L_0)\frac{2\pi}{\omega}\right) - 1} \tag{A4}$$

Let $Z^*(t)$ be the corresponding periodic solution and let $Z(t)$ be any solution. Then $Z^*(t)$–$Z(t)$ converges to zero for t tending to infinity. This proves that any solution of Eq. (3) converges to the periodic solution $Z^*(t)$.

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