# A Model of Pheromone Molecule-Acceptor Interaction

Wolf A. Kafka and Jürgen Neuwirth
Max-Planck-Institut für Verhaltensphysiologie, Seewiesen

(Z. Naturforsch. **30 c**, 278–282 [1975]; received November 8, 1974)

Receptor, Acceptor-sites, Binding Energy, Specificity, Pheromone

**Multiple binding interactions between odor molecules and acceptors are formulated by means of the Boltzmann statistics and intermolecular bond energies. Number, spatial arrangement and electronic properties of binding positions of the acceptor can be defined such that the specificity of sex pheromone receptors in male noctuid moths is quantitatively described.**

## Introduction

The understanding of molecular principles underlaying odor reception has gained considerably from studies of insect olfactory systems [1-3]. Here, electrophysiological methods have permitted to determine the responses of single olfactory receptor cells in regard to stimulus molecules altered, systematically, in shape, flexibility, rotational characteristics, and the positions and nature of functional groups [4-13]. From these studies it has been deduced that weak chemical interactions occur between the odor molecules and proposed types of acceptors on the membrane of the receptor cells. By these interactions the acceptors – presumably by conformational changes – become switched into states which trigger the electrical processes of the cell response [14]. It has further been proposed that the causation of the above (all-or-none) states primarily depends on an appropriate energy transfer between distinct positions of pronounced electron polarisabilities and dipole moments in both binding partners [4, 12, 15]. This step of simplification implies the assumption that equivalent receptor cell responses are initiated by equal numbers of odor molecule-acceptor complexes, regardless of the kind of stimulus compounds. For different compounds the respective stimulus quantities should then reflect the probabilities of forming such complexes.

Quantitative models on chemoreceptive transduction processes have so far focussed on the kinetics rather than on the selectivity of the interaction between stimulus molecules and hypothetical acceptors [16-26]. The molecular details have either been ignored or been expressed only indirectly in terms of equilibrium ocnstants. In contrast to these dynamic models we propose a static model in which the binding probabilities are directly correlated with the molecular characteristics. The model will first be generally formulated; its validity will then be tested by using electrophysiological data from sex pheromone reception in certain species of male Lepidoptera [27-29].

## General Concept of Odor Molecule — Acceptor Interaction

The formalism is directed to correlate electrophysiological activities, binding energies, and certain molecular properties of both the stimulus molecules and hypothetic binding partners (acceptors). First, electron polarisability and dipole moments of a set of stimulus molecules and the electrophysiologically determined activities will be used to calculate the here to fore unknown values of polarisabilities and dipole moments of the acceptor. With these acceptor values defined, the activities of a further set of compounds will then be predicted.

We define $(m)$ as the single compound which revealed to be the most effective in the electrophysiological test. The activity of any other compound $(s)$ will then be expressed by the quotient of the respective stimulus quantities $(c_s, c_m)$, required to elicit an equivalent cell response. This quotient $(c_s/c_m)$ should thus be equivalent to the quotient of the probabilities of the attachment. We explain the probabilities of interaction for the different molecules $(s, m)$ by the Boltzmann-factors

$$\exp(-U_{\text{tot,max}}/k\,T)$$

and the total maximum binding energies $U_{\text{tot,max}}$ between the odor molecule and the acceptor accord-

Requests for reprints should be sent to Dr. Wolf A. Kafka, Max-Planck-Institut für Verhaltensphysiologie, *D-8131 Seewiesen.*

ing to Eqn (1) [15, 30, 31]:

$$\frac{c_s}{c_m} = \frac{\exp({}^{m}U_{\text{tot,max}}/k\,T)}{\exp({}^{s}U_{\text{tot,max}}/k\,T)} = \frac{\exp({}^{m}U_{\text{tot,max}} - {}^{s}U_{\text{tot,max}})}{k\,T} \tag{1}$$

In this equation, changes of entropy, displacements of other molecules from the binding sites, and the possible cooperative interaction between different acceptors are neglected. The binding energy $U_{\text{tot,max}}$ is given by the sum $U_{u,v}$ of the energies between the pronounced binding positions. With $u$ positions in the odor molecule and $v$ positions in the acceptor (subsites), distanced by $r_{u,v}$ and distinguished by distinct electron polarisabilities $a_u$, $a_v$ and dipole moments $p_u$, $p_\text{v}$, the binding energy $U_{\text{tot,max}}$ thus may be expressed by the subsequent Eqn (2) (Fig. 1) [15, 30, 31]:

$$U_{\text{tot,max}} = \sum_{u,v} ({}^{a}U_{u,v} + {}^{p}U_{u,v}), \tag{2}$$

with ${}^{a}U_{u,v} = -3\,J\,a_u\,a_v/4\,r_{u,v}^6$,

and ${}^{p}Uu,v = -f_1\,p_u\,p_v/r_{u,o}^3 - f_2\,p_u^2\,p_v^2/4\,k\,T\,r_{u,v}^6$,

where ${}^{a}U_{u,v}$ = energy of interaction due to electron polarisation; ${}^{p}U_{u,v}$ = energy of dipole interaction; $J$ = Ionizing potential; $k$ = Boltzmann-constant; $T$ = °Kelvin; $f_1$, $f_2$ account for intramolecular rotations of dipoles [30].

Following Eqns (1) and (2), different binding probabilities for various compounds to a distinct acceptor are due to either different positions of functional groups, as expressed by $r_{u,v}$, and/or due to different values of polarisabilities $a_u$ and dipole moments $p_u$. The unknown values of $a_v$, $p_v$ and $r_{u,v}$ will be determined by mathematically adjusting a set of electrophysiological activity values with the values $U_{\text{tot,max}}$ and $(c_s/s_m)$ in Eqns (1) and (2). This process is facilitated if the assumption [4, 5] is accepted that odor molecules interact in their state of lowest energy conformation; then the spatial arrangement of the binding positions (subsites) in the acceptor should correspond to the spatial arrangement of the positions of pronounced electron densities in the most active compound ($m$) at this state.

With structural and electronic properties of the model acceptor thus determined, the same formulae (1, 2) may be applied to predict the activity values for various further compounds. These predicted activities can then be compared to experimental ones, thus testing the validity of the method.

## Application of the Model

In the preceeding paper by Priesner *et al.* [27], electrophysiological data were reported reflecting the specificity of pheromone receptors in male moth (Noctuidae, Lepidoptera) to certain monounsaturated acetates and alcohols. These compounds have in common three positions of pronounced electron densites, *viz.* a terminal methyl group of the hydrocarbon chain ($u = 1$); an ethylene linkage ($u = 2$); and an acetoxy of hydroxy group ($u = 3$), respectively.

Considering receptor cells for which *cis*-9-tetradecen-1-yl acetate is the most active stimulus [27], the respective acceptor thus is characterized by three subsites ($v = 1$, $v = 2$, $v = 3$) with the following mutual distances:

$$\Delta_{v=1,\,v=2} = 7.8\ \text{Å} \qquad \Delta_{v=2,\,v=3} = 13.0\ \text{Å}\,,$$

where segments $1-2$ and $2-3$ include an angle of 154°.

Polarisabilities ($a_{v=1}$, $a_{v=2}$, $a_{v=3}$) and dipole moments ($p_{v=1}$, $p_{v=2}$, $p_{v=3}$) in the hypothetical acceptor were determined by fitting Eqns (1) and (2) in accordance with the electrophysiological activities in the following way (s. also Discussion). The test molecules (a set of monounsaturated actetates varying in chain length and position of the ethylene bond) were defined by coordinates of their binding positions ($v$). In contrast to the three acceptor positions here all intramolecular bonds are included. To take the bearings of the maximum bond energy $U_{\text{tot,max}}$ in Eqn (2), the thus defined stimulus molecules were "moved" in the course of the calculation by means of horizontal translations and rotations parallel to the three positions ($v$) of the hypothetical acceptor at varying distances $d$ (Fig. 1).

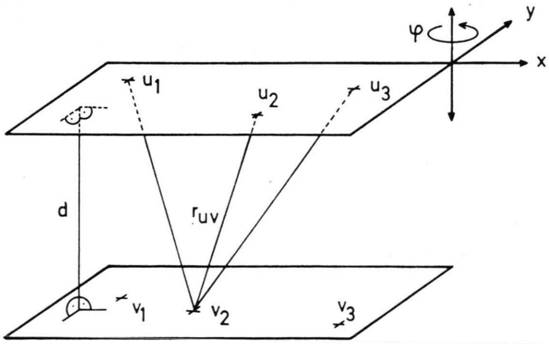

Fig. 1. Mutiple point interaction between $u$ positions of the odor molecule and $v$ positions (subsites) of a model acceptor. Arrows indicate "movements" when tracing $U_{\text{tot,max}}$ [15,30].

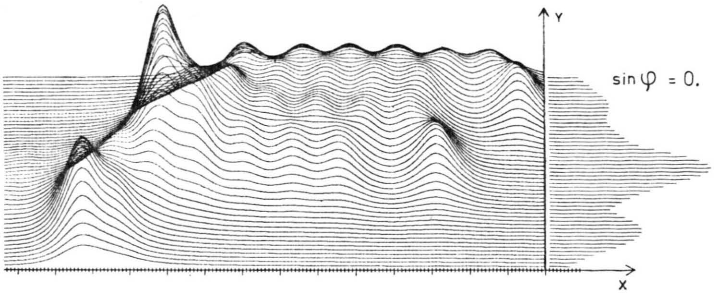

Fig. 2. Total binding energy $U_{\mathrm{tot}}$ between *cis*-9-tetradecen-1-yl acetate and a given model acceptor at 7000 $x,y$-steps. The model acceptor refers to the noctuid moth species *Apamea rubrirena*. Both the $x$- and $y$-movements are in 0.2 Å steps at a constant angle of rotation according to Fig. 1. $U_{\mathrm{tot}}$ is projected in arbitrary units onto the $y$-axis. For each of the 50 $y$-steps the highest value $U_{\mathrm{tot}}$ is projected onto the $x$-axis; one of these renders the maximum total binding energy $U_{\mathrm{tot,max}}$ between the chosen test molecule and the model acceptor. Smaller peaks (*e. g.* the one at far left) arise from binding energies $U_{\mathrm{tot}}$ between less than three $u,v$-combinations according to Fig. 1, whereas the ∼1.5 Å waves in $U_{\mathrm{tot}}$ reflect interactions of the C—H-bonds of the stimulus molecule with the three acceptor positions.

The translation was by steps of 0.2 Å, each followed by rotations in steps 1° from 0 – 90°. Starting with reasonable values for $a_v$, $p_v$ and $d$, the binding energy $U_{\mathrm{tot}}$ was calculated according to Eqn (2) for each step, one of which gave $U_{\mathrm{tot,max}}$ (Fig. 2). Subsequently the values $a_v$, $p_v$ and $d$ were optimized until for the set of compounds used (Fig. 3), the values $(c_s/c_m)$ obtained by Eqn (1) all coincided with the electrophysiological activities reported[27] (Fig. 3).

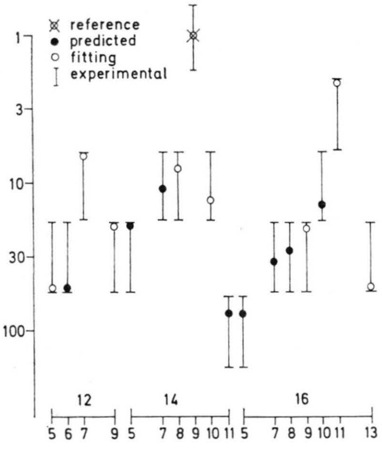

Fig. 3. Activity values of monounsaturated acetates differing in chain length and in position of the *cis*-C=C-bonds (abscissa) for a presumed pheromene receptor of the noctuid moth species *Apamea rubrirena*. Ordinate: numbers of molecules required for a distinct response, *reference* + *cis*-9-tetradecen-1-yl acetate; *experimental* = activity values determined electrophysiologically[27]; *fitting* = activity values resulting in the process of determination of the acceptor values; *predicted* = activity values calculated for further compounds (s. text). All the calculated values are within the class range determined electrophysiologically[27].

A typical set of physico-chemical properties thus ascribed to an acceptor specialized for *cis*-9-tetradecen-1-yl acetate is[30]:

$a_{v=1} = 1.1$ $a_{v=2} = 2.1$ $a_{v=3} = 0.4$ (esu)
$p_{v=1} = -$ $p_{v=2} = -$ $p_{v=3} = 0.7$ (debye)
$d, = 2.5$ Å $f_1, = 0.09$ $f_2, = 0.64$ $J = 9$ (eV)

For this acceptor (which refers to males of the noctuid species *Apamea rubrirena*) the calculated energy of interaction $U_{\mathrm{tot,max}}$ was largely independent from values $p_{v=1}$ and $p_{v=2}$ on account of the small values for $p_{u=1}$ and $p_{u=2}$. The fit was not achieved unless the binding position $v = 1$ (corresponding to the terminal methyl group of the hydrocarbon chain) was shifted up to the plane of the stimulus molecule (in Fig. 1) and 2.1 Å in prolongation of its longitudinal axis. (For a detailed discussion see Neuwirth[30]).

Defining $U_1$, $U_2$, $U_3$ as the binding energies at the position $v = 1, 2, 3$, respectively, their values for the most active compound (*cis*-9-tetradecen-1-yl acetate) were as follows:

$$U_1 = 1.7 \quad U_2 = 7.0 \quad U_3 = 1.5 \quad (1/6.023)\, 10^{-23}\ \text{kcal/molecule}$$

For the other compounds employed in calculations (s. Fig. 3) the corresponding energies ranged between 60 and 90 percent of these values.

By keeping the above acceptor values $a_v$, $p_v$, and $d$ constant, Eqns (1) and (2) were then employed to trace $U_{\mathrm{tot,max}}$ for further molecules (s. Fig. 3), thus calculating their activities towards this acceptor. The obtained values are in perfect agreement with the activities of the electrophysiological measurements (Fig. 3).

## Discussion

Our rather simplified model, here presented, intends to describe the first selective step of the olfactory process, *i. e.* the formation of complexes among oder molecules and hypothetic acceptors on receptor cells in insects. Similar to principles proposed for drug action[23, 32] and recently also for human gustation and olfaction[14, 20, 33–35], these complexes are considered as the product of a joint (cooperative) interaction between more than one binding position in each of the binding partners: A molecule attaching to the acceptor in only one of the acceptor's binding positions, even with higher binding energy, would not get the acceptor switched into a state necessary for cell excitation[14, 36]. It seems even conceivable that bindings which (functional groups of high polarisabilities or dipole moments, *e. g.* COOH, C – C, C = C = C, Br, J. Cl, F) are stronger than those of the most active compounds might prevent the acceptor from switching into the activated state. As an additional possibility, such strong bindings also might prevent conformational changes necessary before the final attachment occurs.

This model was applied to electrophysiological data, reflecting responses of presumed pheromone receptors specialised to *cis*-9-tetradecen-1-yl acetate, a sex pheromone structure often found in moths[24, 27, 29, 37]. As a result of the mathematical fitting process one set of acceptor values only, *i. e.* spatially as well as electronically, was found to reveal binding probabilities of modified compounds fully consistent with their activities determined electrophysiologically. By modifying one or more of these values, this model also seems to be suited to describe the specificities of pheromone receptors reported for related insects having the same or structurally similar pheromones[27–29].

The energies $U_1$, $U_2$ and $U_3$ transferred at the subsites (see Eqn (2)) must be considered to comprise both an attraction and an activation component. The calculated energy thus may not reflect the actual values transferred in the process of activating the acceptor. It is reasonable to assume that proportions of these energies $U_1$, $U_2$ and $U_3$ (especially those of the far-ranging and strong bindings) are involved in guiding the stimulus molecules to the binding sites, and keeping them in the acceptor field for a certain minimum of time. Considering the Heisenberg Uncertainty Principle[38] and the total binding energies ($\sim 10^{-23}$ kcal per molecule), this minimum of time should be in the range of $10^{-14}$ – $10^{-13}$ sec. These energies are in the range of weak chemical interactions.

The molecules considered here had identical functional groups, but at different spatial arrangements. This reveals that different binding energies are due to different distances $r_{u,v}$ between the interacting positions (Eqn (2)). With increasing the distances, the attracting field of the acceptor becomes more homogeneous; with respect to the binding energies differences in chain length or in positions of functional groups should thus be of more significance for compounds acting at smaller distances $r_{u,v}$ (*i. e.* "well fitting" molecules), and of less significance for compounds acting at increased distances. In fact when in the electrophysiological measurements a compound was 100 or 1000 times less active than the most active one, various further small structural modifications resulted in almost no further change in activity[27, 39].

One would also expect that there are molecular properties which have almost the same influence on the activity of different molecules in a homologous series, *e. g.* the intramolecular rotations and flexibilities of *cis*- and *trans*-isomers. The respective compounds considered here, monounsaturated acetates have quite different shapes when in their lowest energy conformation. However rotation of C – C bonds would enable a *trans*-compound to attain the bent shape, similar to its *cis*-isomer. Therefore, if a *trans*-compound in this bent state, interacts with an acceptor specialized to a *cis*-compound, the activity ratio between the respective *cis*- and *trans*-isomers should depend mainly on this bending and thus remain almost the same during a shift of the double bond or an elongation of the carbon chain. This is indeed the pattern found by electrophysiological measurements[27, 29].

It should be noted that the values of dipole moments and electron polarisabilities, calculated for the hypothetic acceptor, are of reasonable physical magnitude for functional groups[40].

We thank Prof. Dr. D. Schneider, Prof. Dr. H. Z. Levinson, Prof. Dr. H. Bestman, Dr. E. Priesner, Dr. E. Kramer, Dr. R. A. Steinbrecht for valuable criticism and comments.

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