C(S)

Peripheral Mechanisms of Pheromone Reception in Moths

Karl-Ernst Kaissling

Max-Planck-Institut für Verhaltensphysiologie Seewiesen, 82319 Starnberg, Germany

Correspondence to be sent to: K-E. Kaissling, Max-Planck-Institut für Verhaltenphysiologie Seewiesen, 82319 Starnberg, Germany

Abstract

Moths pheromones mostly consist of two or a few chemical components in a species-specific ratio. Each component is perceived by a particular type of receptor cell. Some pheromone components can inhibit the behavioral responses to other pheromone components. A single pheromone molecule is sufficient to elicit a nerve impulse. The dose–response curve of single pheromone receptor neurons increases over many decades of stimulus intensity. Pheromone receptor cells can resolve single stimulus pulses up to a frequency of 10 pulses/s. Electrophysiological and biochemical studies on perireceptor events suggest that the pheromone molecules interact with the receptor cell while bound to a reduced form of the pheromone binding protein. The enzymatic degradation of pheromone found on the antennae is much too slow to account for the decline of the receptor potential after end of stimulation. The postulated rapid deactivation of the odor molecules adsorbed might be performed by an oxidation of the pheromone binding protein. Several second messenger systems seem to be involved in the cellular transduction mechanism (IP_3, diacylglycerol, cGMP, Ca^{2+}). It is, however, not excluded that pheromone molecules can gate single ion channels directly and thus elicit the elementary receptor potentials, observed at weak stimulus intensities. **Chem. Senses 21: 257–268, 1996.**

Introduction

Many aspects of chemoreception have been studied in insects since the first insect pheromone has been chemically identified in the silkmoth *Bombyx mori* (Butenandt *et al.*, 1991). This paper will select a few general topics and present examples from work on a few insect species which might be of relevance also for other groups of animals. We describe the coding of stimulus quality, stimulus intensity and the temporal pattern of stimulation. Furthermore, olfactory transduction in pheromone receptor cells including perireceptor events and intracellular messenger cascades will be discussed.

Coding of pheromone quality

In the last three decades, hundreds of female and male pheromone components have been chemically identified in numerous species and in most insect families (Arn *et al.*, 1992). The choice of *Bombyx mori* for isolating and analysing the first female sex lure was fortunate because the behavioral response of the male used as a bioassay can be elicited by a single pheromone component (*E,Z*-10,12-hexadecadien-1-ol,bombykol) (Schneider, 1992). This compound excites one of the two receptor cells innervating each of the 17 000 long olfactory hairs (sensilla trichodea) on one male antenna (Kaissling and Priesner, 1970; Steinbrecht, 1973).

It turned out that *Bombyx* was an exception where a single pheromone component elicits the full sexual behavior of the male, ranging from wing vibration over anemotactic walking to the readiness to copulate upon contact with a female. In most lepidopteran species the female pheromone is a mixture of several components of which two or more are obligatory and have to be present in a certain species specific proportion in order to attract the conspecific male (Priesner, 1986; see also Masson and Mustaparta, 1990). Thus, species communicating within the same habitat can utilize the same substances if they are mixed in different proportions. Often species being attracted by the same chemicals are not closely related taxonomically (Figure 1). For instance from about 4500 lepidopteran species that are native to Central Europe, the number of their chemically distinct pheromone components totals only about 150 (Arn *et al.*, 1992).

It seems a general rule also true for other orders of insects, e.g. bark beetles, that each pheromone component acts on a particular type of highly-specific receptor cell tuned to this component (Masson and Mustaparta, 1990). This means that every alteration of the pheromone molecule reduces its effectiveness by a factor of 10, up to 1000 times or more. A pheromone blend elicits a particular excitation pattern across the different types of receptor cells which is evaluated by the CNS (Boeckh and Ernst, 1987; Homberg *et al.*, 1989). Only the correct pattern of excitations elicited by the species-specific pheromone blend leads to a successful behavior of the male.

In some species only the simultaneous excitation of all cell types, i.e. the suprathreshold concentration of all obligatory pheromone components elicits a behavioral response (Linn *et al.*, 1986). In other species each pheromone component alone can elicit a different type of behavior (like anemotactic flight, landing, walking, etc.) which in a correct sequence, enables the male to find the female (for cockroach see Seelinger and Gagel, 1985).

Often there is one major component in the pheromone blend released by the female (Masson and Mustaparta, 1990). Surprisingly, the male is more sensitive to this compound than to the minor pheromone components. This can be demonstrated in two closely related species of Saturniid moths (*Antheraea polyphemus* and *A. pernyi*) which use the same three pheromone components, but with different major components (Meng *et al.*, 1989) (Figure 2). The behavioral sensitivity of these moths has not been measured; its maximum value can, however, be inferred from the number of receptor cells (N) and their measured sensitivities (S_{cell}). In both species the product $N \times S_{cell}$ is

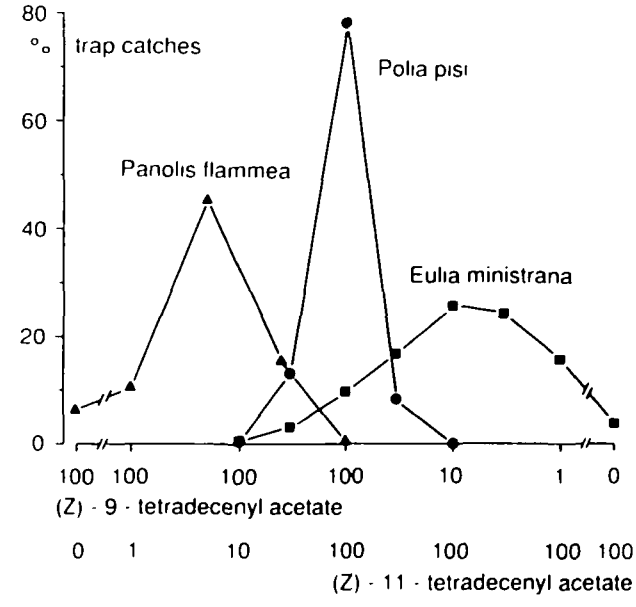
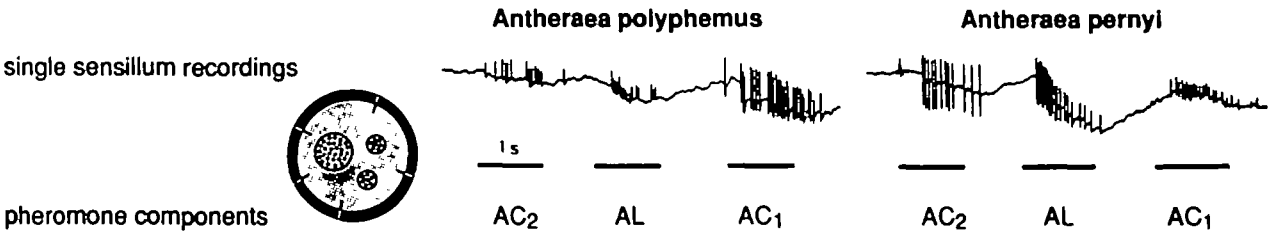


Figure 1 *Attraction of male moths to various mixtures of two pheromone components* (abscissa). Data from trap experiments with the pine beauty moth, *Panolis flammea* (Priesner *et al.*, 1978), another noctuid moth, *Polia pisi* (Priesner, 1980b) and the leaf-roller species *Eulia ministrana* (Priesner, 1984) The males of *P. pisi* have four types of pheromone receptor cells two of which respond to the two pheromone components. The remaining two receptor cells are tuned to (*Z*)-11-hexadecenyl acetate and (*Z*)-7-dodecenyl acetate These compounds are pheromone components of other species of moths and inhibit the attraction of *P. pisi* males when added to the *P pisi* pheromone (Priesner, 1986).

maximal for the major component released in highest quantity by the female (relative release rate R). Taking all three factors into account the major pheromone component ought to be perceived by the male moth over a much larger distance ('active space') than the other components (Figure 2). This suggests that the major component alone must have a specific effect on male behavior.

Curiously, the extracellularly recorded nerve impulses often show largest peak-to-peak amplitudes in the cell responding to the major component. Whether this characteristics which also has been found in many other species has a functional significance is highly questionable, but not disproven. It has been suggested that the receptor cell with the thicker dendrite in the hair produces the larger nerve impulses (Hansson *et al.*, 1994a). This seems unlikely for the case of *Antheraea pernyi* because the sensilla trichodea have only one thicker dendrite, whereas two of the three receptor cells show large nerve impulses (Figure 2).

Pheromone components also can inhibit behavioral responses whereas they excite a particular type of receptor neuron. This was found for the second pheromone component of *Bombyx mori*, (*E,Z*)-10,12-hexadecadienal (bombykal),



rel. numbers of cells	N	25	83	100	20	100	102
rel. sensitivities of cells	S_{cell}	15	75	100	38	100	75
rel. sensitivities of moth	**N x S_{cell}**	**4**	**62**	**100**	**8**	**100**	**77**
rel. release rates	R	n. d.	11	100	40	100	20
rel. active spaces	**R x N x S_{cell}**	**n. d.**	**7**	**100**	**3**	**100**	**15**

Figure 2 Potential relative sensitivities ($N \times S_{cell}$) of two species of Saturniid moths of their three pheromone components AC_1 = (*E,Z*)-6,11-hexadecadienyl acetate, AC_2 = (*E,Z*)-4,9-tetradecadienyl aceate and AL = (*E,Z*)-6,11-hexadecadienal The sensitivity values ($N \times S_{cell}$) are multiplied by the estimated release rates (*R*) of the respective components (Bestmann *et al*, 1987) and give a rough measure of the relative distances (relative active spaces) over which the respective compounds can be perceived. These distances are largest for the major pheromone component (AC_1 in *A. polyphemus* and AL in *A. pernyi*). All numbers are given as a percentage of the values for the major component of each species Above, left. schematic cross-section of one of the 60 000 olfactory hairs (sensilla trichodea) per antenna with one thicker and two thinner dendrites of three receptor cells (same in both species!) (Keil, 1984). Above. electrophysiological recordings from one sensillum of each species stimulated with the three pheromone components in a sequence. The AC_1-cell delivers large nerve impulses in *A. polyphemus*, but small ones in *A. pernyi*, the other two cells produce small nerve impulses in *A. polyphemus* and large ones in *A. pernyi* (data from Meng *et al.*, 1989)

which has been detected by electrophysiological recordings from the second olfactory cell in the sensillum trichodeum after testing many candidate stimulus compounds (Kaissling *et al.*, 1978). Bombykal was the most effective compound and could afterwards be isolated from the female gland (Kasang *et al.*, 1978); it is released by the female moth in a small (10%) proportion to bombykol. Its behavioral effect is a partial inhibition of wing vibration and other responses of the male to bombykol; maximally, bombykal can shift the behavioral threshold towards 1000-fold higher concentrations of bombykol. The inhibition takes place by central comparison of the messages coming from both types of receptor cells.

The significance of the inhibition of male behavior by bombykal is not yet known. However, in many other species it is known that pheromone components of one species may inhibit the attraction of a sympatric species (allomonal interruption, Birch *et al.*, 1980; see also Mustaparta, 1996, this issue). This interspecific signal excites separate receptor neurons which are tuned to the inhibitory compound (first found in bark beetles, Mustaparta *et al.*, 1977; for moths see Priesner, 1980a).

All receptor cells tuned to pheromone components (including the behavioral inhibitors) send their axons to a specific region of the antennal lobe, the macroglomerular complex (MGC, see Mustaparta, 1996, this issue). Each type of pheromone receptor cell can project to a different subdivision of the MGC (Hansson *et al.*, 1994b). The axons of receptor cells responding to general odors terminate in the ordinary glomeruli of the antennal lobe.

Coding of stimulus intensity

One pheromone molecule is sufficient to elicit a nerve impulse as was shown for the male silkmoth *Bombyx mori* (Kaissling and Priesner, 1970). At the behavioral threshold the pheromone concentration was about 1000 molecule/cm^3 at an airstream velocity of 60 cm/s and with a stimulus duration of 1 s. The flux of pheromone molecules was on the average about 4 molecules per 100 hairs per second (eliciting about 1 nerve impulse/100 cells/s). This means that the male shows a behavioral reaction (e.g. wing vibration) if about 1% of the bombykol receptor cells fire one nerve impulse per second in addition to the spontaneous firing. With a spontaneous rate of 0.1 nerve impulses per cell and per second the CNS is able to detect within 1 s a signal of

170 pheromone-elicited nerve impulses from a noise of 1700 nerve impulses fired by the 17 000 cells of one antenna (Kaissling, 1987).

This extremely efficient signal to noise detection must take place in the MGC of the antennal lobe where all axons from pheromone receptor cells converge to secondary neurons. The convergence factor in terms of cell numbers and also in sensitivity (Boeckh and Boeckh, 1979) between receptor and secondary neurons in the MGC is between 100 and 1000. With a convergence factor of 100 a secondary neuron would receive an input of 10 spontaneous nerve impulses per second. At the behavioral threshold an input of one nerve impulse would be added. A similar convergence factor is found in the olfactory bulb of vertebrates suggesting similar neural mechanisms for improving signal contrast. According to threshold considerations, vertebrate olfactory neurons should also be able to respond with impulse firing to single odor molecules (Kaissling, 1990).

Pheromone receptor neurons respond over a wide range of stimulus intensities. The rate of nerve impulses fired by a cell increases linearly with the flux of stimulus molecules until the cell fires a few impulses per second. At higher stimulus intensities the impulse response changes from a tonic response to a phasic-tonic response with an initial burst and a subsequent lower level of impulse rate; at very high intensities, a purely phasic burst of impulses is elicited, followed by a silent period without impulse firing (Kaissling, 1986a).

The peak rate of nerve impulses increases over several decades of stimulus intensities up to 300 impulses per second with a slope below 0.3 in a double log plot (Figure 3). The dose–response curves of the individual receptor cells are somewhat steeper and they vary with respect to their position along the intensity axis (by less than a factor of 10) (Zack, 1979; see also Almaas and Mustaparta, 1991; Mustaparta, 1996, this issue). This means that, with increased stimulus concentration, receptor neurons that are less sensitive are recruited.

In the example of *A. polyphemus* (Figure 3) an increase of response can be observed even above a flux of 10^7 pheromone molecules per hair and per second. Such stimulus intensities might occur naturally only if the male hits the pheromone gland with its antennae; afterwards the cell response is strongly adapted and needs minutes to disadapt and hours for full recovery.

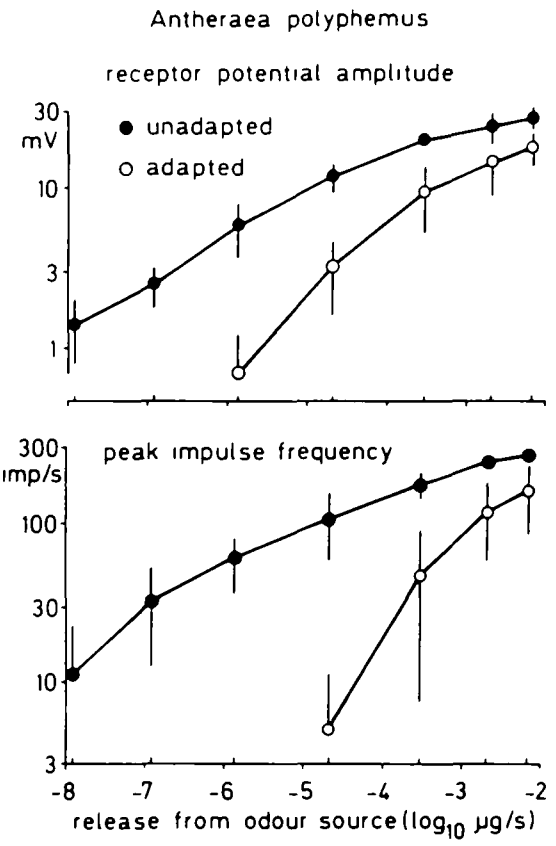


Figure 3 Dose–response relationships obtained from 10 receptor cells tuned to the pheromone component (*E,Z*)-6,11-hexadecadienyl acetate (AC_1) (from Zack, 1979) The receptor potential amplitude was measured 2 s from begin of stimulation The peak impulse frequency was determined from 10 consecutive intervals between nerve impulses in each response. The abscissa was calibrated using the ^{3}H-labelled stimulus compound (AC_1) (Kaissling *et al*, 1987). The stimuli were given in a sequence from lower to higher stimulus strengths. The measurements were repeated (circles) 60 s after the maximum stimulus which served as an adapting stimulus. At the lowest stimulus strength a sensillum adsorbed about 30 pheromone molecules per s (K-E Kaissling and S. Kanaujia, unpublished)

Temporal coding

It was first shown by Kramer (1986) that the anemotactic approach of an insect to the odor source consists of several turns into the upwind direction per second (Figure 4). Each turn is elicited by a brief (<100 ms) odor pulse caused by encountering the pheromone-containing air filament originating from the female gland. As expected from these behavioral studies, the pheromone receptor cells and even higher order neurons within the antennal lobe (Christensen and Hildebrand, 1988) are able to respond to pulsed stimuli of several pheromone pulses per second (Figure 5). The time resolution measured depends on the type of receptor cell (Rumbo and Kaissling, 1989; Almaas *et al.*, 1991) and reaches values up to 10 odor pulses per second; the astonishing resolution is restricted to higher stimulus intensities where the response latency is below 100 ms with a minimum latency of about 10 ms (Kaissling and Boekhoff, 1993). The responses in the range of single molecule detection have an

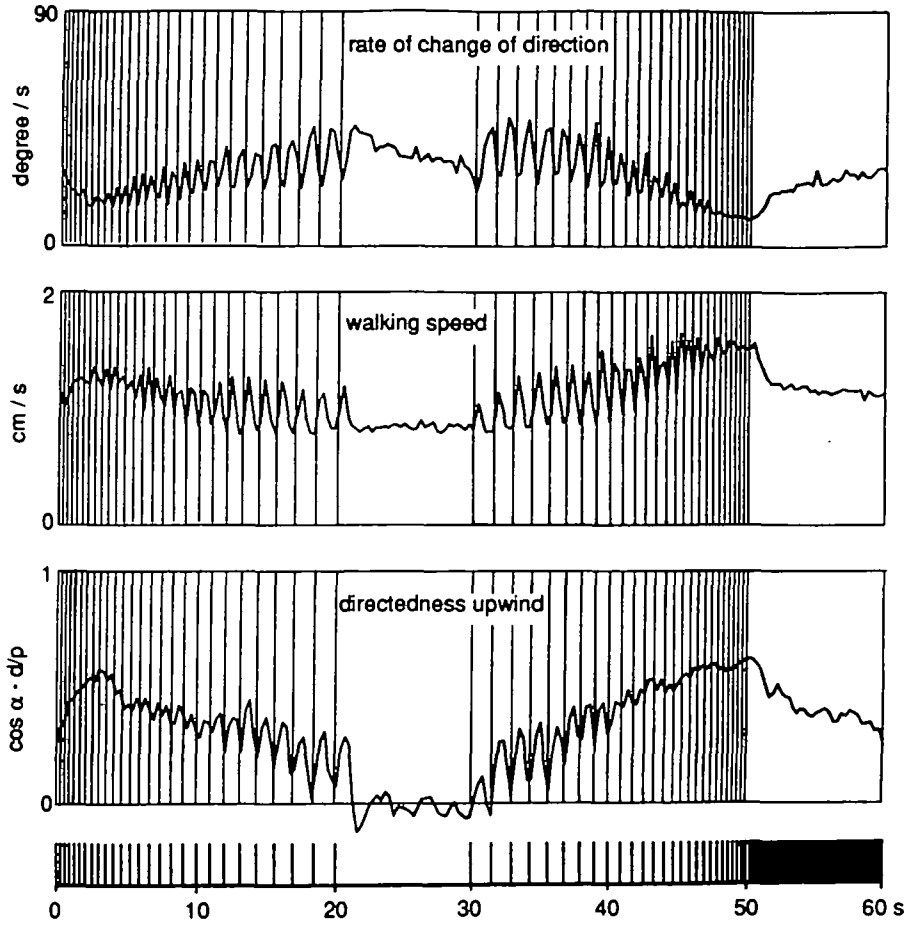


Figure 4 Anemotactic responses of walking male moths of *Bombyx mori* elicited by pulsed stimuli of bombykol (vertical bars, pulse duration 150 ms). Stimulus pulses were first given with increasing intervals followed by a 10-s pause; then pulses were given with decreasing intervals followed by a 10-s period of permanent stimulation This sequence was repeated 10 times for each run The behavioral responses were measured by means of a locomotion compensator ('Kramer sphere') and averaged over 55 runs of altogether 12 animals. Three different parameters were evaluated with a time resolution of 0 1 s. rate of change of direction, walking speed and directedness upwind. The latter is the product cos $\alpha \times d/p$ (α = course angle with respect to the wind direction, $\alpha = 0$ represents the true upwind course, d is the distance and p the path length of the walk). The anemotactic orientation is best at highest frequency of bombykol pulses, clearly better than at permanent stimulation (Kramer, 1986).

average latency of several 100 ms with a large scatter (Kaissling and Priesner, 1970; Kaissling, 1986b). Correspondingly, the time resolution at low stimulus intensities must be impaired.

Perireceptor events

In some insect species the antennae are constructed as very effective molecular sieves. The large antennae of saturniid moths quantitatively cleanse the air of pheromone molecules. According to studies with radiolabelled pheromone about 80% of the adsorbed molecules are caught by the long hairs of the sensilla trichodea and penetrate the hair wall (Kanaujia and Kaissling, 1985). Finally, the radioactivity can be found in the sensillum lymph filling the hair lumen and bathing the outer dendritic segments of the one to three pheromone receptor cells innervating the hair. The extracellular sensillum lymph is equivalent to the mucus covering the olfactory cells in vertebrates (Pelosi, 1994). This fluid not only provides the ionic milieu (200 mM K^+, 40 mM Na^+; Kaissling and Thorson, 1980) for the outer dendritic segments of the receptor cells, it also contains enzymes degrading the pheromone, and the highly concentrated pheromone binding protein (PBP) (5–10 mM, Vogt and Riddiford, 1981; 15 kD, 142 amino acids, Raming *et al.*, 1989). The PBP specifically occurs in the sensilla trichodea innervated by pheromone receptor cells (Figure 6).

Perireceptor events such as the transport of the stimulus molecules from the hair surface towards the receptor cells and the removal of active pheromone molecules from the receptors might govern the time course of the receptor potential and thus be important for the transduction of the

pheromone stimulus into a nerve impulse response. The decline of the receptor potential after the end of stimulation occurs within seconds; it could reflect the arrival of stimulus molecules at the receptor cell delayed by diffusional transport within the hair. Its time characteristics and also the latencies of the nerve impulses elicited by single pheromone molecules, i.e. at weak stimulus intensities (several hundred ms), are compatible with a minimum apparent diffusion coefficient of 5×10^{-9} cm^2/s for the pheromone transport towards the receptor cell (Kaissling, 1987). However, a much higher apparent diffusion coefficient (3×10^{-7} cm^2/s) was determined for the transport of ^3H-labelled pheromone in a longitudinal direction on the hair (Kanaujia and Kaissling, 1985). This suggests that the pheromone transport towards the receptor cell is relatively quick and a slower process governs the time course of the receptor potential.

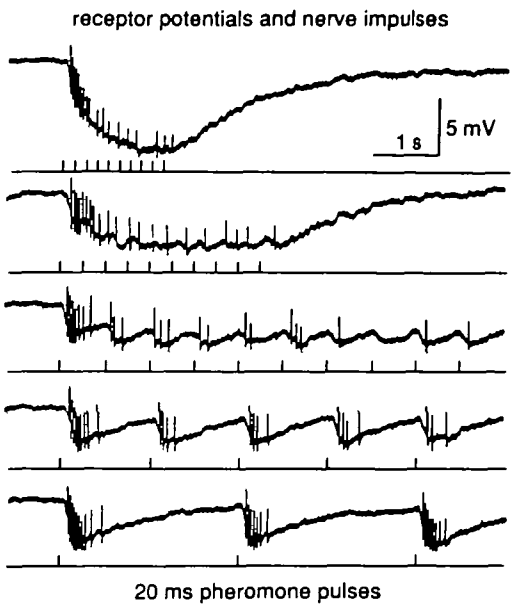


Figure 5 Responses recorded from a single sensillum of *Antheraea polyphemus* to 20 ms pulses of the pheromone component (*E,Z*)-6,11-hexadecadienal presented at various time intervals. The responses are produced by one receptor cell at a temperature of 8°C; the responses to single stimulus pulses fuse at a frequency of about 3 pulses/s (Kodadová, 1993). At higher temperature this cell type resolves up to 10 pheromone pulses/s (Rumbo and Kaissling, 1989). Note in all traces but the lowest one that the nerve impulse response adapts after the first stimulus; it is almost recovered after 3 s (as can be seen in the lowest trace).

Three processes of stimulus removal seem too slow to account for the receptor potential decline: (i) the desorption of pheromone molecules from the antenna (20% of the adsorbed molecules desorb from the antenna within 30 min); (ii) the decrease of pheromone concentration on the hairs due to migration of the molecules towards the antennal branches (half life on the hairs 3 min; Kanaujia and Kaissling, 1985); and (iii) the enzymatic pheromone degradation (half life of chemically intact pheromone on the antennae is 3 min; Kasang *et al.*, 1988).

The velocity of pheromone degradation *in situ* is much slower than the one calculated from degradation experiments with purified sensillar esterase (Vogt *et al.*, 1985). This discrepancy could be resolved considering the finding that the degradation proceeds more slowly in the presence of PBP (Vogt and Riddiford, 1986). If the PBP protects the pheromone from degradation, in the sensillum lymph only

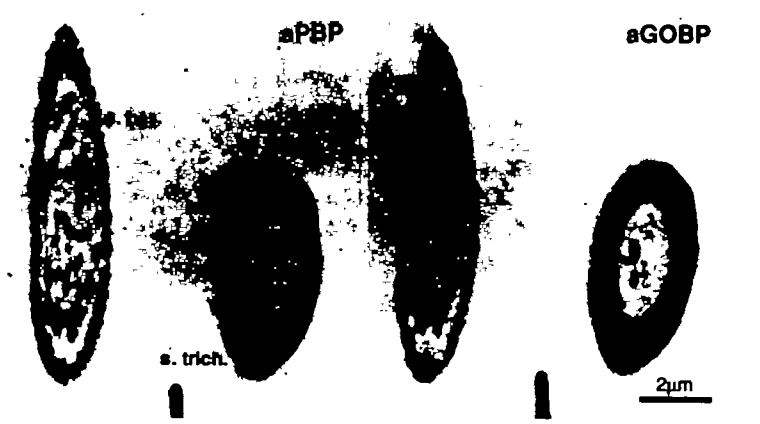


Figure 6 Cross-sections of two olfactory hairs of *Antheraea polyphemus* (*S. basiconicum* and *S. trichodeum*) in two subsequent ultra-thin sections each of which was treated with a different antibody. Secondary antibodies were labelled with gold particles. On the left: the anti-PBP antibody stained the sensillum trichodeum, innervated by pheromone receptor cells. On the right: the sensillum basiconicum with receptor cells responding to general odorants was stained with the antibody against the so-called general odorant binding protein. By courtesy of M. Laue, see Laue *et al.* (1994).

the small fraction of free pheromone concentration ($S_{free}/S_{bound} = K_d/PBP_{tot}$) is degraded by the enzyme (Kaissling, 1986b, 1987). This fraction is $6.4 \cdot 10^{-5}$ if one uses PBP_{tot} = 10 mM (Vogt and Riddiford, 1981; Klein, 1987) and the dissociation constant of the pheromone-PBP complex determined for the recombinant PBP (oxidized form, K_d = 640 nM; Du *et al.*, 1994). The dissociation constant of the PBP isolated from antennae, probably mostly in its reduced form, was 60 nM (determined by J. Hemberger in a different type of assay, see Kaissling *et al.*, 1985).

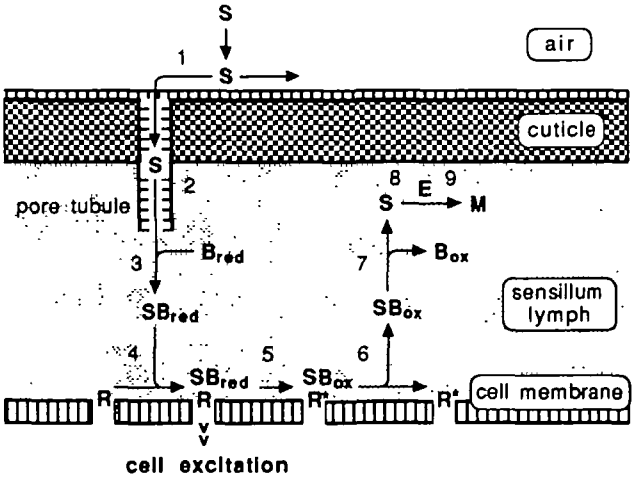
A mechanism of rapid pheromone deactivation, such as required for the receptor potential decline, has been suggested from biochemical studies on the PBP in the moth *Antheraea polyphemus* (Ziegelberger, 1994, 1995).

The PBP occurs in two forms, the oxidized form with three intramolecular disulfide bridges (PBP_{ox}) and a partially reduced form with one or two disulfide bridges (PBP_{red}). PBP obtained from unstimulated antennae contains about 80% of PBP_{red} and 20% of PBP_{ox}. Pheromone is bound to both forms. In homogenates of isolated hairs containing PBP and receptor cell dendrites the relative amount of PBP_{red} associated with pheromone decreases and the relative amount of pheromone-PBP_{ox} increases with longer incubation time. In control experiments with purified PBP (without cellular material) the ratio PBP_{red}/PBP_{ox} associated with pheromone remains constant. This suggests that the complex pheromone-PBP_{red} changes into pheromone-PBP_{ox} in the presence of receptor cell dendrites. Possibly the redox shift of the PBP is mediated by the receptor molecules. The working hypothesis was proposed that the pheromone bound to PBP_{ox} is not able to stimulate further receptor molecules. Thus, the redox shift could serve as a rapid deactivation mechanism for the pheromone which allows that new stimuli are still able to excite the cell (Ziegelberger, 1994, 1995).

The question needs to be answered whether the velocity of the redox shift is high enough to account for the receptor potential decline. *In vitro*, 12% of PBP_{red} were converted into PBP_{ox} within 10 min. The life time of active pheromone *in situ* can be estimated from the decline of the receptor potential (Zack, 1979) if one considers the non-linear relationship between receptor potential amplitude and the concentration of active pheromone in the sensillum lymph. Assuming that this relation is approximately given by the (static) dose–response curve (Figure 3) the half life of the active pheromone in the sensillum would be between 0.5 s at lower, up to 2 s at higher stimulus intensities (Kaissling, 1995 and unpublished). Considering the different experimental conditions, including the dilution of the homogenate by more than 200 fold compared with the situation in the living hair, the pheromone-PBP_{red} complex could be converted into pheromone-PBP_{ox} within a much shorter time, sufficient for the postulated pheromone deactivation. The present state of hypotheses about the perireceptor events is summarized in Figure 7.

Cellular transduction

Since practically all pheromone is bound to the PBP (see above) it is most likely the complex pheromone-PBP_{red} which stimulates the receptor cell (Van den Berg and Ziegelberger, 1990). The dissociation constant of the complex and the putative receptor ought to be around 100 µM (or higher) because this is the pheromone concentration in the hair at the upper end of the dose–response curve (Figure 3), where it is not yet fully saturated. This value suggests much weaker binding of the pheromone-PBP_{red} complex to the receptor than between pheromone and PBP_{red} (K_d = 60 nM, see above).



S - pheromone
M - pheromone metabolite
B - pheromone binding protein
R - receptor
E - pheromone degrading enzyme

Figure 7 Schematic diagram of perireceptor events in an insect olfactory hair. (1) Surface diffusion of pheromone molecules (S). (2) Diffusion through pore tubules. (3) Binding of pheromone to the reduced form of PBP (B_{red}). (4) Interaction of the complex SB_{red} with the putative receptor molecule (R) in the receptor cell membrane leading to excitation of the receptor cell. (5) Redox-shift of the PBP-pheromone complex. (6) Dissociation of the complex SB_{ox} from the receptor. (7) Dissociation of the complex SB_{ox}. (8 and 9) Enzymatic degradation of the pheromone forming an inactive metabolite (M). According to a working hypothesis, the pheromone bound to the oxidized PBP is deactivated, i.e. not able to activate further receptors (Ziegelberger, 1994, 1995).

Antheraea polyphemus	pmole/mg protein	µM inside conc.	references
IP_3			
basal level in antennal homogenate	80	19.3 (calc.)**	Kaissling and Boekhoff 1993
25 ms after 1 pM pheromone	400		
i. e. increase by	320	590 (calc.)$	
no ion channel opening in patch		1 (appl.)	Zufall and Hatt 1991
basal level in intact antenna, male	141 +/- 58	34.0 (calc.)**	Ziegelberger 1991
female	204 +/- 101	49.2 (calc.)**	
after 10 s pheromone stimulus	no increase		
DOG, analog of DAG			
ion channel opening in patch		0.36 (appl.)	Zufall and Hatt 1991
cAMP			
basal level in intact antenna	14.2 +/- 2.9	3.43 (calc.)**	Ziegelberger et al. 1990
after 10 s pheromone stimulus	no increase		
cGMP			
basal level in intact antenna	3.0 +/- 0.6	0.7 (calc.)**	Ziegelberger et al. 1990
increase after 1 - 60 s pher. stim.	+ 34%	+3.1 (calc.)§	
ion channel opening in patch		1 (appl.)	Zufall and Hatt 1991
K for 1/2 open probability		55 (appl.)	
block of CAN channels in patch		10 (appl.)	Zufall et al. 1991

(calc.) = calculated using 50 µg of protein per antenna

(appl.) = applied concentrations in patch clamp experiments

** = unpubl., calculated assuming equal concentration in all (sensory and non-sensory cells) of the antenna

$ = unpubl., average conc. increase within the receptor cell dendrite (48µm^3) following one pheromone hit

§ = unpubl., concentration increase related to volume of receptor cell soma + inner dendrite (Ziegelberger et al 1990)

Figure 8 Summary of quantitative studies on second messengers in the moth *Antheraea polyphemus*. The intracellular concentrations (µM inside concentration) were calculated using morphometrical studies of Keil (1984) and Gnatzy *et al.* (1984). The extraordinary high increase of IP_3 in the antennal homogenate is not understood. The pheromone stimulus was the major component (*E,Z*)-6,11-hexadecadienyl acetate.

Activation of the putative receptor molecules finally leads to increased ion conductance of the dendritic cell membrane (Kaissling and Thorson, 1980; Zufall and Hatt, 1991; Hatt and Zufall, 1993). As also found in crustaceans (Fadool and Ache, 1992), several intracellular signal compounds appear to be involved in transduction altogether in a single cell. As an example, all effects of second messengers (such as 1,4,5 inositol triphosphate, diacyl glycerol, cGMP and Ca^{2+}) as observed in intact antennae, antennal homogenates and membrane patches of isolated receptor cells are summarized for one insect species in Figures 8 and 9. However, it cannot be excluded that, in addition, ion channels can be opened directly, perhaps via G-proteins (Brown and Birnbaumer, 1990).

More than one type of ion channel seem to contribute to the graded receptor potential and further channels must be involved in the generation of nerve impulses in the soma and axon region of the receptor cell. There is evidence that a pheromone stimulus initiates a sequence of production of different second messengers acting on several types of ion channels as suggested for pheromone receptor cells in the moth *Manduca sexta* (Stengl, 1994). In *Antheraea polyphemus* the initial burst of nerve impulses observed at higher stimulus intensities might be induced by opening of a Ca^{2+} activated non-specific ion (CAN) channel located in the soma region of the receptor cell (Zufall *et al.*, 1991). This phasic response adapts very quickly (see Figure 5) which might be due to the fact that this type of channel is blocked by cGMP.

Extracellular recordings under 'loose patch' conditions *in situ* (0.2 GΩ seal between neighbouring hair sensilla) at weak stimulus concentrations have revealed 'elementary receptor potentials' which precede single nerve impulses and are the first electrical events elicited by single pheromone

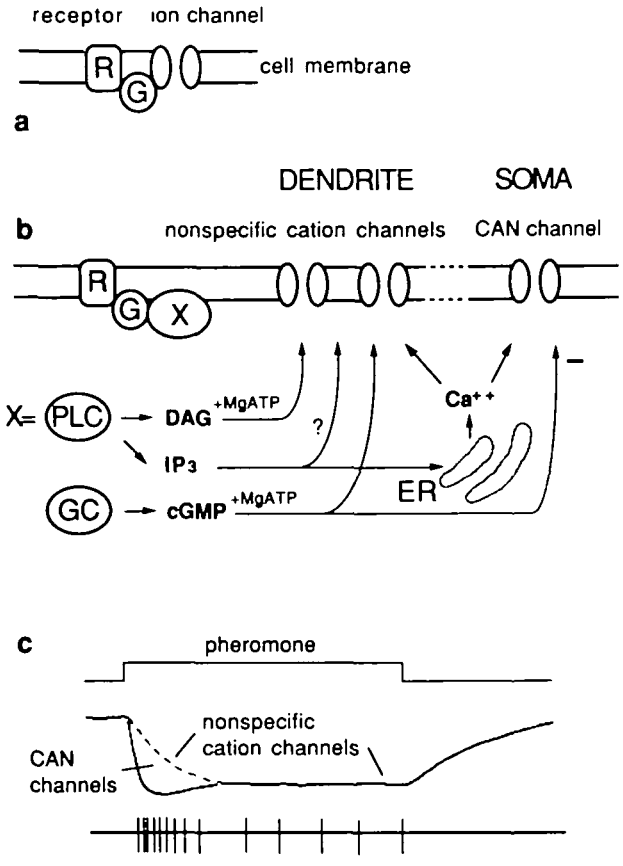


Figure 9 Schematic diagram of possible second messenger effects on ion channels of pheromone receptor cells of the moth *Antheraea polyphemus* (for references see Figure 8) (a) Direct opening of ion channels The receptor molecules (R) could be directly coupled with ion channels via G-proteins. (b) Effects of second messengers on various types of ion channels in dendrite and soma membrane of the receptor cell 'Boosting' effect of Ca^{2+}-activated nonspecific (CAN) ion channels enhancing the initial phase of the receptor potential (suggested by Zufall *et al*, 1991).

molecules (Figure 10) (Kaissling, 1977, 1994). According to an analysis of the electrical equivalent circuit of the sensillum they are produced by an increase of dendritic membrane conductance in the range of 30 pS (Kaissling and Thorson, 1980). Consequently, these transient potential changes of 0.2–0.5 mV height and 10–50 ms duration could represent openings of single ion channels. Channel openings of about 50 pS were observed upon stimulation of inside out patches with cGMP or a diacylglycerol analogue, in the presence of MgATP (Figure 8). However, it is not excluded that the conductance increase of 30 pS observed *in situ* is due to several smaller channel openings which open synchronously.

Final remarks

In each of the areas discussed interesting problems are still unsolved.

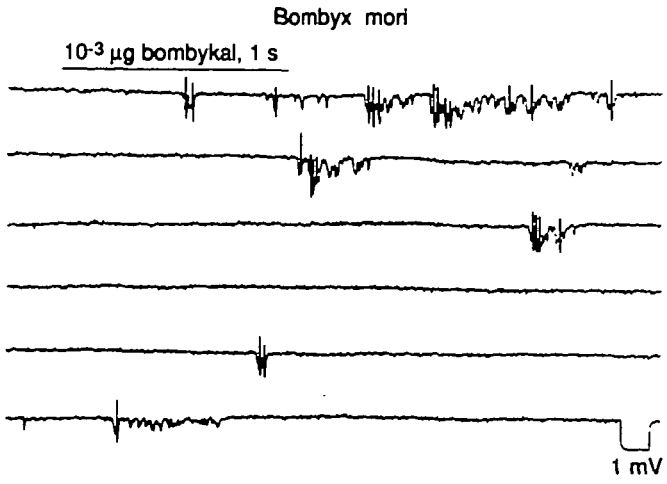


Figure 10 Response of a pheromone receptor cell of a male moth of *Bombyx mori* to a weak 1-s stimulus of bombykal (10^{-3} µg per filter paper). The continous recording for 18 s shows a sequence of elementary receptor potentials (ERP) which are thought to be elicited by single pheromone molecules (Kaissling, 1994). They occur as single transient deflections or as bursts, sometimes superimposing on each other. Not all of them are followed by nerve impulses The time delay between stimulus and responses is relatively long in the example shown. On the average the delay is a few hundred ms. According to an analysis of the electrical circuit of the sensillum the ERP reflects a conductance increase of the dendritic membrane in the range of 30 pS (Kaissling and Thorson, 1980). This corresponds to opening of single ion channels found in patch clamp recordings (Zufall and Hatt, 1991) However, it cannot be excluded that an ERP is produced by several channels of smaller conductance

More thorough behavioral analysis is required to describe the role of major and minor pheromone components including the mechanism of behavioral inhibition.

The mechanism is unknown which produces the shallow slope of the dose–response curve which enables the receptor cell to dynamically respond over a wide range of stimulus intensities. Several possibilities have been discussed, also for the increased slope of the dose response curve due to adaption (Figure 3) (Kaissling, 1986b, 1987). Another challenging task is to study the mechanism of the signal-to-noise detection in the macroglomerular complex.

Based on behavioral observations a mathematical model has been proposed which describes anemotactic behavior in walking and flying moths (Kramer, in press). This model helps to analyse the behavioral effects of odor stimuli in a more quantitative manner. Furthermore, it is little understood which processes govern the dynamic properties of the receptor cells, as reflected in the receptor potential as well as the nerve impulse response. It has to be clarified whether perireceptor events or second messenger cascades dominate the time course of the receptor potential. An urgent project is to test the working hypothesis of pheromone deactivation

via the proposed oxidation of the complex PBP-pheromone. Many questions are open with respect to the cellular transduction mechanism, e.g. the question how the elementary receptor potentials are generated.

It is fascinating not only to analyse particular mechanisms in the olfactory system, but also to study their interactions and their adaptation to the specific biological functions.

REFERENCES

Almaas, T.J. and Mustaparta, H. (1991) Heliothis virescens: Response characteristics of receptor neurons in sensilla trichodea type 1 and type 2. *J. Chem. Ecol.*, **17**, 953–972.

Almaas, T.J., Christensen, T.A. and Mustaparta, H. (1991) Chemical communication in Heliothine moths. 1. Antennal receptor neurons encode several features of intraspecific and interspecific odorants in the male corn-earworm moth *Helicoverpa zea*. *J. Comp. Physiol.*, **169**, 249–258.

Arn, H., Tóth, M. and Priesner, E. (1992) *List of Sex Pheromones of Lepidoptera and Related Attractants*, 2nd edn. OILB Publications, Montfavet

Bestmann, H-J., Attygalle, A.B., Brosche, T., Erler, J., Platz, H., Schwarz, J., Vostrowsky, O., Wu, C.H., Kaissling, K-E. and Chen, T.-M. (1987) Identification of three sex pheromone components of the female Saturniid moth *Antheraea pernyi* (Lepidoptera: Saturniidae). *Z. Naturforsch.*, **42c**, 631–636.

Birch, M.C., Light, D.M., Wood, D.L., Browne, L.E., Silverstein, R.M.L., Bergot, B.J., Ohloff, G., West, J.R. and Young, J.C. (1980) Pheromonal attraction and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. *J. Chem. Ecol.*, **6**, 703–717.

Boeckh, J. and Boeckh, V. (1979) Threshold and odor specificity of pheromone-sensitive neurons in the deutocerebrum of *Antheraea pernyi* and *A. polyphemus* (Saturnidae). *J. Comp. Physiol.*, **132**, 243–255.

Boeckh, J. and Ernst, K.D. (1987) Contribution of single unit analysis in insects to an understanding of olfactory function. *J. Comp. Physiol.*, **161**, 549–565.

Brown, A.M. and Birnbaumer, L. (1990) Ionic channels and their regulation by G protein subunits. *Ann. Rev. Physiol.*, **52**, 197–213.

Butenandt, A., Beckmann, R. and Stamm, D. (1961) Über den Sexuallockstoff des Seidenspinners. II. Konstitution und Konfiguration des Bombykols. *Hoppe Seylers Z. Physiol. Chem.*, **324**, 84–87.

Christensen, T.A. and Hildebrand, J.G. (1988) Frequency coding of central olfactory neurons in the sphinx moth *Manduca sexta*. *Chem. Senses*, **13**, 123–130.

Du, G., Ng, C-S. and Prestwich, G.D. (1994) Odorant binding by a pheromone binding protein: activation site mapping by photoaffinity labeling. *Biochemistry*, **33**, 4812–4819.

Fadool, D.A. and Ache, B.W. (1992) Plasma membrane inositol 1,4,5-triphosphate-activated channels mediate signal transduction in lobster olfactory receptor neurons. *Neuron*, **9**, 907–918.

Gnatzy, W., Mohren, W. and Steinbrecht, R.A. (1984) Pheromone receptors in *Bombyx mori* and *Antheraea pernyi*, II. Morphometric analysis. *Cell Tissue Res.*, **235**, 35–42.

Hansson, B.S., Hallberg, E., Löfstedt, C. and Steinbrecht, R.A. (1994a) Correlation between dendrite diameter and action-potential amplitude in sex-pheromone specific receptor neurons in male *Ostrinia nubilalis* (Lepidoptera, Pyralidae). *Tissue Cell*, **26**, 503–512.

Hansson, B.S., Ljungberg, H., Hallberg, E. and Löfstedt, C (1994b) Functional specialization of olfactory glomeruli in a moth. *Science*, **16**, 1313–1315.

Hatt, H. and Zufall, F. (1993) Molecular components underlying signal transduction in olfaction. *Verh. Dtsch. Zool Ges.*, **86.2**, 95–109.

Homberg, U., Christensen, T.A. and Hildebrand, J.G. (1989) Structure and function of the deutocerebrum in insects. *Ann. Rev. Entomol.*, **34**, 477–501.

Kaissling, K-E. (1977) Structures of odour molecules and multiple activities of receptor cells. In Le Magnen, J. and MacLeod, P. (eds), *International Symposium on Olfaction and Taste VI*. Information Retrieval, London, pp. 9–16.

Kaissling, K-E. (1986a) Temporal characteristics of pheromone receptor cell responses in relation to orientation behaviour of moths. In: Payne, T.L., Birch, M.C. and Kennedy, C.E.J. (eds), *Mechanisms in Insect Olfaction*. Oxford University Press, Oxford, pp. 193–200.

Kaissling, K-E. (1986b) Chemo-electrical transduction in insect olfactory receptors. *Ann. Rev. Neurosci.*, **9**, 121–145.

Kaissling, K-E. (1987) In Colbow, K. (ed.), *R. H. Wright Lectures on Insect Olfaction*. Simon Fraser University, Burnaby, B.C., Canada.

Kaissling, K-E. (1990) Antennae and noses: their sensitivities as molecule detectors. In Torre, V., Cervetto, L. and Borsellino, A. (eds), *Sensory Transduction*. Plenum Press, New York and London, pp. 81–97.

Kaissling, K-E. (1994) Elementary receptor potentials of insect olfactory cells. In Kurihara, K., Suzuki, N. and Ogawa, H. (eds),

International Symposium on Olfaction and Taste XI. Springer Verlag, Tokyo, pp. 812–815.

Kaissling, K-E. (1995) Pheromone deactivation on the antenna of the moth *Antheraea polyphemus*: its minimum velocity and its possible mechanism. In Elsner, N. and Menzel, R. (eds), *Proceedings of the 23rd Göttingen Neurobiology Conference II.* Thieme, Stuttgart, Abstract 362.

Kaissling, K-E and Boekhoff, I. (1993) Transduction and intracellular messengers in pheromone receptor cell of the moth *Antheraea polyphemus.* In Wiese, K., Gribakin, F.G., Popov, A.V. and Renninger, G. (eds), *Sensory Systems of Arthopods.* Birkhäuser Verlag, Basel, pp. 489–502.

Kaissling, K-E. and Priesner, E (1970) Die Riechschwelle des Seidenspinners. *Naturwiss,* **57**, 23–28.

Kaissling, K-E. and Thorson, J. (1980) Insect olfactory sensilla: Structural, chemical and electrical aspects of the functional organization. In Sattelle, D B., Hall, L.M and Hildebrand, J.G. (eds), *Receptors for Transmitters, Hormones and Pheromones in Insects.* Elsvier/North-Holland, Amsterdam, pp 261–282.

Kaissling, K-E., Kasang, G., Bestmann, H.J., Stransky, W. and Vostrowsky, O. (1978) A new pheromone of the silkworm moth *Bombyx mori.* Sensory pathway and behavioral effect. *Naturwiss.,* **65**, 382–384.

Kaissling, K-E., Klein, U., de Kramer, J.J , Keil, T.A., Kanaujia, S. and Hemberger, J. (1985) Insect olfactory cells: electrophysiological and biochemical studies. In Changeux, J-P., Hucho, F., Maelicke, A. and Neumann, E. (eds), *Molecular Basis of Nerve Activity. Proceedings of the International Symposium in Memory of D. Nachmansohn.* De Gruyter, Berlin, pp 173–183.

Kaissling, K-E., Zack Strausfeld, C. and Rumbo, E (1987) Adaptation processes in insect olfactory receptors: mechanisms and behavioral significance. *International Symposium on Olfaction and Taste IX, Ann. N.Y. Acad. Sci.,* pp. 104–112.

Kanaujia, S. and Kaissling, K-E (1985) Interactions of pheromone with moth antennae: Adsorption, desorption and transport. *J. Insect Physiol.,* **31**, 71–81.

Kasang, G., Kaissling, K-E., Vostrowsky, O. and Bestmann, H.J. (1978) Bombykal, a second pheromone component of the silkworm moth *Bombyx mori. Angew. Chem. Int. Ed. Engl.,* **17**, 60.

Kasang, G., von Proff, L. and Nicholls, M. (1988) Enzymatic conversion and degradation of sex pheromone in antennae of the male silkworm moth *Antheraea polyphemus. Z. Naturforsch.,* **43c**, 275–284.

Keil, T.A. (1984) Reconstruction and morphometry of silkmoth olfactory hairs: a comparative study of sensilla trichodea on the antennae of male *Antheraea polyphemus* and *Antheraea pernyi* (Insecta, Lepidoptera). *Zoomorph.,* **104**, 147–156.

Klein, U. (1987) Sensillum lymph proteins from antennal olfactory hairs of the moth *Antheraea polyphemus* (Saturniidae). *J. Insect Biochem.,* **17**, 1193–1204.

Kodadová, B. (1993) Effects of temperature on the electrophysiological response of moth olfactory sensilla. Dissertation, Institute of Experimental Phytopathology and Entomology of the Slovakian Academy of Sciences, Bratislava 1993.

Kramer, E. (1986) Turbulent diffusion and pheromone triggered anemotaxis In Payne, T.L., Birch, M.C. and Kennedy, C.E.J. (eds), *Mechanisms in Insect Olfaction.* Oxford Unviersity Press, Oxford, pp. 59–67.

Kramer, E. (1996) A tentative intercausal nexus and its computer model on insect orientation in windborne pheromone plumes. In Cardé, R. and Minks, A (eds), *Pheromone Research: new directions.* Chapman & Hall, New York (in press)

Laue, M., Steinbrecht, R.A. and Ziegelberger, G. (1994) Immunocytochemical localization of general odorant binding protein in olfactory sensilla of the silkmoth *Antheraea polyphemus. Naturwiss,* **81**, 178–180.

Linn, C.E., Jr, Campbell, M.G and Roelofs, W.L. (1986) Male moth sensitivity to multicomponent pheromones: Critical role of female-released blend in determining the functional role of components and active space of the pheromone *J. Chem. Ecol.,* **12**, 659–668.

Masson, C. and Mustaparta, H. (1990) Chemical information-processing in the olfactory system of insects. *Physiol. Rev.,* **70**, 199–245.

Meng, L.Z., Wu, C.H , Wicklein, M. Kaissling, K-E. and Bestmann, H.J. (1989) Number and sensitivity of three types of pheromone receptor cells in *Antheraea pernyi* and *Antheraea polyphemus. J. Comp. Physiol.,* **165**, 139–146.

Mustaparta, H., Angst, M.E. and Lanier, G.N. (1977) Responses of single receptor cells in the pine engraver beetle, *Ips pini* (Say) (Coleoptera: Scolytidae) to its aggregation pheromone, ipsdienol, and the aggregation inhibitor, ipsenol. *J. Comp. Physiol.,* **121**, 343–347.

Pelosi, P. (1994) Odorant-binding proteins. *Crit. Rev. Biochem. Molec. Biol.,* **29**, 199–228.

Priesner, E. (1980a) Sensory encoding of pheromone signals and related stimuli in male moths. In: *Insect Neurobiology and Insecticide Action (Neurotox 79).* Society of the Chemical Industry, London, pp. 359–366.

Priesner, E. (1980b) Sex attractant system in *Polia pisi* L. (Lepidoptera: Noctuidae). *Z. Naturforsch.,* **35c**, 990–994.

Priesner, E. (1984) The pheromone receptor system of male *Eulia ministrana* L., with notes on other Cnephasiini moths. *Z. Naturforsch.,* **39c**, 849–852.

Priesner, E. (1986) Correlating sensory and behavioral responses in multichemical pheromone systems of Lepidoptera. In Payne,

T.L. Birch, M. and Kennedy, C. (eds), *Mechanisms in Insect Olfaction*. Oxford University Press, Oxford, pp. 225–233.

Priesner, E., Bogenschütz, H., Altenkirch, W. and Arn, H (1978) A sex attractant for the pine beauty moth. *Panolis flammea. Z. Naturforsch.*, **33c**, 1000–1002.

Raming, K., Krieger, J. and Breer, H. (1989) Molecular cloning of an insect pheromone-binding protein. *FEBS Lett.*, **256**, 215–218.

Rumbo, E.R and Kaissling, K-E. (1989) Temporal resolution of odour pulses by three types of pheromone receptor cells in *Antheraea polyphemus. J. Comp. Physiol. A*, **165**, 281–291.

Schneider, D. (1992) 100 years of pheromone research, an essay on Lepidoptera. *Naturwiss.*, **79**, 241–250.

Seelinger, G. and Gagel, S. (1985) On the function of sex pheromone components in *Periplaneta americana:* improved odour source localization with periplanone A. *Physiol. Entomol.*, **10**, 221–234.

Steinbrecht, R.A. (1973) Der Feinbau olfaktorischer Sensillen des Seidenspinners (Insecta, Lepidoptera): Rezeptorfortsätze und reizleitender Apparat. *Z. Zellforsch. mikrosk. Anat.*, **139**, 533–565.

Stengl, M. (1994) Inositol-triphosphate-dependent calcium currents precede cation currents in insect olfactory receptor neurons *in vitro. J. Comp. Physiol. A*, **174**, 187–194.

Van den Berg, M.J. and Ziegelberger, G. (1991) On the function of the pheromone binding protein in the olfactory hairs of *Antheraea polyphemus. J. Insect Physiol.*, **37**, 79–85.

Vogt, R.G. and Riddiford, L.M. (1981) Pheromone binding and inactivation by moth antennae. *Nature*, **293**, 161–163.

Vogt, R G. and Riddiford, L.M. (1986) Pheromone reception: a kinetic equilibrium. In Payne, T.L., Birch, M.C. and Kennedy, C.E.J. (eds), *Mechanisms in Insect Olfaction* Clarendon Press, Oxford, pp. 201–208.

Vogt, R G., Riddiford, L.M. and Prestwich, G D. (1985) Kinetic properties of a pheromone degrading enzyme: the sensillar esterase of *Antheraea polyphemus. PNAS*, **82**, 8827–8831.

Zack, C. (1979) Sensory adaptation in the sex pheromone receptor cells of saturniid moths. Dissertation, Fakultie Biologie, Ludwig-Maximilians-Universität, München.

Ziegelberger, G. (1991) Cyclic nucleotides in the olfactory system of the moths *Antheraea polyphemus* and *Bombyx mori*. In Doving, K. (ed.), *International Symposium on Olfaction and Taste X*, GCS A/S, Olso, pp. 85–91.

Ziegelberger, G. (1994) The odorant binding protein in the silkmoth *Antheraea polyphemus*. In Elsner, N and Breer, H. (eds), *Sensory Transduction, Proceedings of the 22nd Göttingen Neurobiology Conference I.* Thieme, Stuttgart, p. 86

Ziegelberger, G. (1995) Redox shift of the pheromone-binding protein in the silkmoth *Antheraea polyphemus. Eur. J. Biochem.*, **232**, 706–711.

Ziegelberger, G., Van den Berg, M.J., Kaissling, K-E , Klumpp, S. and Schulz, J.E. (1990) Cyclic GMP levels and guanylate cyclase activity in pheromone sensitive antennae of the silkmoth *Antheraea polyphemus* and *Bombyx mori. J. Neurosci.*, **10**, 1217–1225.

Zufall, F. and Hatt, H. (1991) Dual activation of sex pheromone-dependent ion channel from insect olfactory dendrites by protein kinase C activators and cyclic GMP. *Proc. Nat. Acad Sci USA*, **88**, pp. 8520–8524.

Zufall, F., Hatt, H. and Keil, T.A. (1991) A calcium-activated nonspecific cation channel from olfactory receptor neurons of the silkmoth *Antheraea polyphemus. J. Exp. Biol.*, **161**, 455–468.