SPECIAL ISSUE

PHYSIOLOGY OF PHEROMONE RECEPTION IN INSECTS (an example of moths)

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Introduction

Pheromones, chemical signals for intraspecific communication *(1)*, are usually blends of chemical compounds in species-specific mixtures. Airborne insect pheromones often consist of only two or three chemicals each of which is perceived by a specific type of receptor cell, called an olfactory specialist. A specialist cell is tuned to one compound, its key compound, and can also respond when that compound has been slightly modified, if it is presented at 10- to 1000-fold higher stimulus concentrations. Thus the composition of a pheromone blend is represented by the pattern of excitations across the types of specialist cells *(2)*. In many insects, the responses of individual specialist cells can be easily recorded by extracellular electrodes. Insect antennae - combined sense organs including the function of noses - provide simple and convenient subjects for morphological and biochemical studies. Due to limitations of space this review will cover various aspects of pheromone reception in a few species of moths only. Work in moths and several other model insects is described in numerous reviews and books *(3 - 14)*. Studies of pheromone reception enhance our general knowledge on chemoreception as well as provide a basis of applications for pest control *(15 - 17)*.

Production and biological function of pheromones

Except for contact pheromones insect pheromones are volatile chemicals produced by a large variety of glands located at various places in the insect body. For instance, the sex attractant bombykol (E,Z)-10, 12- hexadecadiene-1-ol) *(18, 19)* is secreted by the abdominal sacculi laterales *(20)* of the female silkmoth *Bombyx mori* (Fig. 1), together with

Fig. 1. Female *Bombyx mori* in calling position, with everted abdominal pheromone glands.

traces of the (E,E)-isomer of the alcohol *(21)* and the analogous (E,Z)-aldehyde bombykal *(22)*. Bombykol alone is able to elicit a pattern of sexual behaviour of the male moth (Fig. 2), such as wing vibration, walking, and turning so that it is headed upwind. The excitatory effect of bombykol is partially blocked if bombykol is presented together with bombykal *(23)*. In fact bombykal may elevate the threshold concentration for bombykol up to 1000-fold. The inhibition occurs by central processing of the excitatory responses of bombykol and bombykal receptor cells. The biological function of the inhibition is not known. In other species of moths, however, the sexual behaviour may be blocked by a pheromone component of a different but usually closely related moth species. Interestingly the moth whose behavior is blocked may possess a specialist receptor-cell type tuned to the behavioural inhibitor.

One example for such a behavioural inhibition is provided

Fig. 2. Male Bombyx mori in alerted position, with combed antennae elevated. By courtesy of R.A. Steinbrecht.

by the gypsy and nun moths *(Lymantria dispar, L. monacha)* which live sympatrically in parts of Europe and share (+)-disparlure as attractant (24). In addition to the attractant the female nun moth produces (-)-disparlure which keeps the male gypsy moth from approaching her. The male gypsy moth has two receptor cells specialized for different ones of the two enantiomers. The male nun moth, however, does not have receptor cells for the inhibitor and is attracted by females of both species.

Many pheromones of female moths are straight-chain unsaturated hydrocarbons with a terminal alcohol, aldehyde, or acetate function and are synthetized in the epithelium of the abdominal glands (see list of sex pheromones of Lepidoptera, 'Pherolist', 25). Usually a pheromone consists of more than one component. Often two or three (or more) components are attractive in a certain species-specific ratio where the relative amounts of a synergistic component can be less than 0.1 %. The same components mixed in a different ratio might represent the pheromone of a different species *(25)*. Many female moths are not able to smell their own pheromone, but there are exceptions *(26)*. E. g. males and females of *Spodoptera littoralis* smell the female pheromone; they learned to extend their proboscis upon pheromone stimuli *(27)* similar as honey bees upon the pheromone component 9-oxo dec-2-enoic acid *(28)*.

Males of several insect orders, mainly lepidopteran species, e. g. of the danaine butterflies or arctiide moths release pheromones serving as aphrodisiacs *(29)*. These odours are often distributed from expandable scent organs called 'androconia' (Fig. 3), for instance brushes of cuticular hairs which produce fine (3 - 5 µm) particles impregnated with the pheromone (Fig. 4). This 'love dust' sticks on the antenna of the females providing a long lasting source of the stimulus that makes the female receptive to copulation *(30)*. The aphrodisiacs of Danainae originate from plant alkaloids, which are actively sought out, taken up and metabolized by the male *(31)*. Pheromonal compounds such as danaidone,

Fig. 3 Males of the milkweed or monarch butterfly *Tirumala petiverana* (Danainae) (left hand) and the asian arctiide moth *Creatonotos gangis* (right hand) with expanded androconia. During its mating flight the monarch male hovers above the female and sprays the pheromone-impregnated 'love dust' onto her antennae. The male Arctiide hanging on a twig attracts both sexes in the evening with its inflated odour tubes. By courtesy of M. Boppré

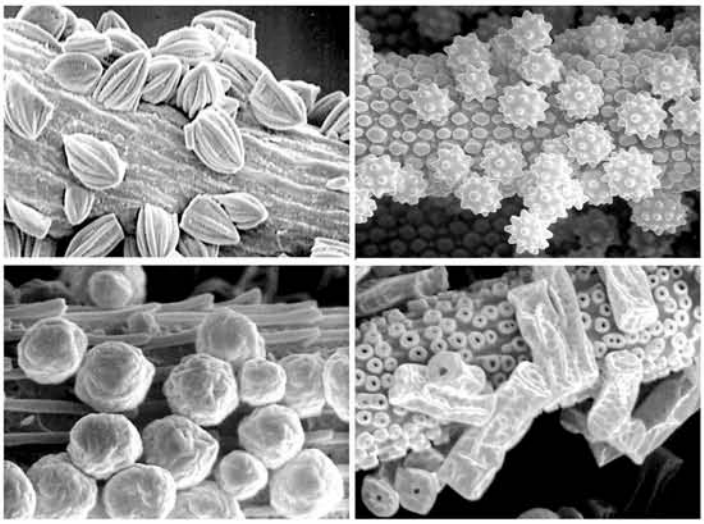

Fig. 4 'Love dust' particles on the hairs of the androconia of the male danaine butterflies Parantica sita, Danaus formosa (upper row), Danaus sp. and Amauris tartarea (bottom row). The particle size is 3 - 5 µm. By courtesy of M. Boppré

hydroxydanaidal, and danaidal, frequently used by Danainae and Arctiidae, and often occurring as blends of two components are derived from pyrrolizidine alkaloids (PAs). PAs ingested by the larvae from their host plants regulate both scent organ morphogenesis and pheromone biosynthesis in the arctiide moth genus *Creatonotos (32, 33)*.

Communication by pheromones is highly developed in social insects such as honeybees, ants, or termites, which bear numerous pheromone glands on various body parts and produce a variety of chemicals. Correspondingly they possess

a large number of types of specialist receptor cells *(34, 35)*. Pheromones of social insects are not only involved in sexual behaviour but also serve as attractants or repellents of either gender of conspecifics, as markers of food, of trails, as recognition signals and have many other functions. For example the honeybee queen *(Apis mellifera L.)* produces pheromones that function in roles such as attracting a retinue of workers around her, attracting drones on mating flights, preventing workers from reproducing at the individual (worker egg-laying) and colony (swarming) level, and regulating several other aspects of colony functioning *(36)*. The queen produces a synergistic, multiglandular pheromone blend of at least nine components for retinue attraction. In Termites a blend of cuticular hydrocarbons may play a key role in colony recognition *(37)*. Differences in the composition of cuticular hydrocarbons among colonies form the most important variable explaining variation in aggression between colonies.

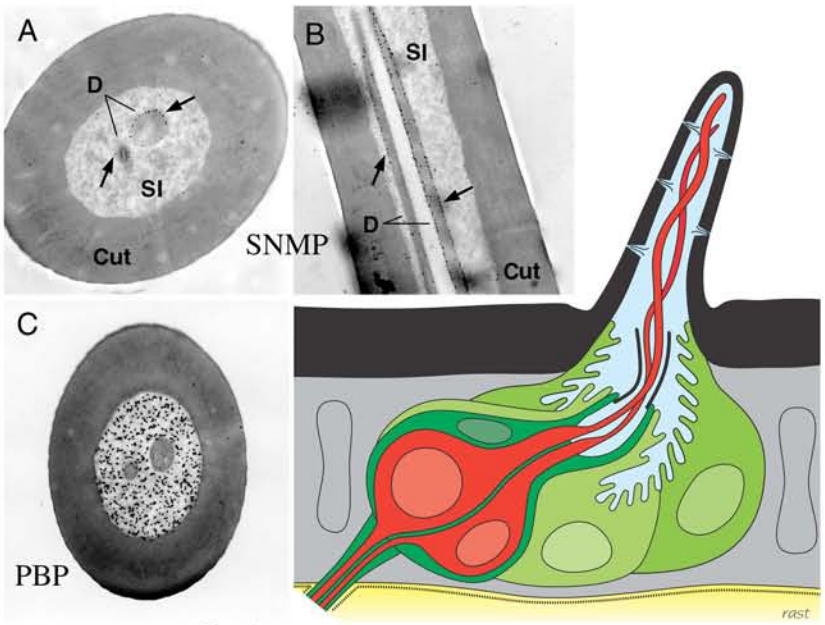

Fig. 6. Right hand: Scheme of an olfactory sensillum trichodeum with two receptor cells (red) and three auxiliary cells (green). In blue: sensillum lymph. A,B: Olfactory hairs of *Antheraea polyphemus:* Electronmicrographic sections treated with gold-labeled antibodies against SNMP (119). The gold particles are associated with the membrane of the receptor cell dendrites (D). Sl = sensillum lymph, Cut = cuticle. C: Gold particles attached to antibodies against PBP show even distribution of the protein within the sensillum lymph (96). By courtesy of R.A. Steinbrecht

Morphology of insect olfactory organs

Insect pheromone receptor cells are located on the antennae among other types of olfactory receptor cells but also receptor cells for stimuli of other sensory modalities (Fig. 5). In contrast to the vertebrate nasal epithelium different types of receptor cells are not randomly

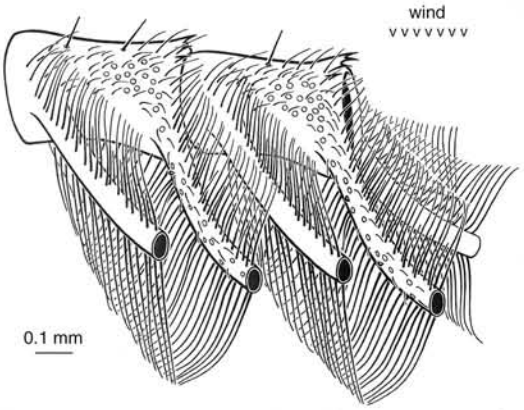

Fig. 5. Antenna of a male saturniid moth, central part of two antennal segments with several types of sensilla. The long hairs, sensilla trichodea, are innervated by two or three pheromone receptor cells. The shorter hairs and pegs (circles) respond to plant odours. There are three bristles (above) for taste and mechanical stimuli, and organs for humidity and temperature (above, looking like claws, pointing to the right-hand side). From (4).

intermingled but grouped in olfactory sensilla, which are morphologically and physiologically well-defined units *(38 - 40)*. They typically consist of hollow cuticular hairs (10 to 400 µm long, 1 to 5 µm thick) innervated by one or several olfactory receptor cells and furnished with three auxiliary cells (Fig. 6). The distal processes (olfactory dendrites, 0.1 to 0.5 mm in diameter) of the receptor cells extend into the hair lumen, and their axons connect to the antennal lobe of the central nervous system (CNS) *(41, 42)*, the insect equivalent of the vertebrate olfactory bulb. During ontogeny the auxiliary cells produce the cuticular wall of the hairs (or sometimes plates). Later they withdraw from the hair lumen and secrete the sensillum lymph. This extracellular medium bathing the olfactory dendrites corresponds to the mucus covering the vertebrate olfactory epithelium. Besides odorant-binding proteins, and pheromone-degrading enzymes (see below) it contains an unusual ion composition (200 mM K^+, 40 mM Na^+) *(43, 44)*. Between sensillum lymph and hemolymph there is a transepithelial potential of +40 mV, produced by an electrogenic pump located in the folded apical membrane of auxiliary cells. The receptor-cell dendrites have a ciliary portion about 2 µm long that separates the inner dendritic segment from the outer. The outer segment contains no

cellular organelles except microtubules. In shorter hairs it is often branched, with at least one microtubule per branch. The cuticle of olfactory hairs or plates is penetrated by typical pore tubules (10 nm in diameter), extending into the hair lumen and rarely contacting the dendritic cell membrane *(45)*. Odour molecules adsorbed on the sensillum surface reach the pores and cross the hair wall, most likely by diffusing through the pore tubules.

Sensilla housing pheromone receptor cells often have longer hairs than sensilla with cells for general (= non-pheromone) odours. Longer hairs provide a more efficient capture of stimulus molecules per receptor cell. In cases of two or three pheromone components the respective sensilla house two or three specialist cells, one for each of the components *(46)*. Receptor cells for pheromone components and behavioral inhibitors can occur in the same sensillum. With several thousands of such sensilla covering the antenna a very fine spatial resolution of the pheromone distribution in air is feasible. It has been shown experimentally that the fine-scale distribution of pheromone components or of the pheromone blend and behavioural inhibitors influences the orientation of flying males approaching an odour source *(47)*. It should be noted that antennae represent 'everted' noses, which enable the insect to detect spatial odour patterns. Thus a topical representation of antennal areas was found in the central nervous system of cockroach *(48)*.

Electrophysiology

A simple approach with which to investigate stimulus-response characteristics is the electroantennogram (EAG), which represents summed fractions of receptor potentials of many olfactory sensilla located near both of the electrode tips inserted into the antenna *(24, 49, 50)*. The so-called receptor potential reflects a change in membrane potential and may be recorded extra- or intracellularly. The EAG is particularly useful for measuring the responses of the odor specialists, the type of receptor cells tuned to a specific key compound. EAG recordings and also recordings from single sensilla combined with gas-chromatography have been employed to identify the effective components of blends, either of pheromones or of plant volatiles *(51)*. Olfactory sensilla, especially the long sensilla trichodea, allow transepithelial recording of receptor potentials and nerve impulses from the two or three identified receptor cells innervating the sensillum. Electrical contact can be obtained by slipping the recording electrode capillary over the cut hair tip (Fig. 7). With a special way of cutting *(50)*, the receptor-cell dendrites are severed but immediately sealed, a method which avoids short-circuiting the membrane potential. This method of recording allows some of the sensillum lymph in the hair to be replaced by the fluid inside the recording capillary. For instance, pheromone

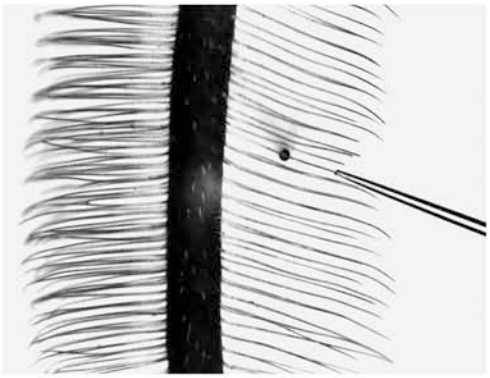

Fig. 7. Side branch of a saturniid antenna. A glass capillary of the recording electrode is slipped over one of several cut hairs. Another capillary is directed to the middle of the hair for applying local stimuli.

may be dissolved in the electrode electrolyte and directly applied to the receptor-cell dendrite inside the hair *(52, 53)*.

Pheromone sensilla with long hairs are convenient subjects for studying the electrical organization of the sensillum circuit *(43, 54 - 57)*. Recording from the tip of a cut hair provides conditions equivalent to those for loose patch-clamp recordings; in this case, the entire dendrite represents the patch of the receptor-cell membrane. Typically the transepithelial resistance is around 200 MΩ and the sensilla are well isolated from each other. The analysis revealed a high specific resistance of the dendritic membrane (3000 Ωcm^2) providing a length constant of the dendrite large enough to conduct a distal membrane depolarization to the cell soma region and to elicit nerve impulses. Pheromone stimuli elicit negative receptor potentials (= negative deflections of the transepithelial potential) up to 30 mV and nerve impulses of a few mV starting with a positive deflection (Figs. 8, 9). These polarities suggest an initial slow depolarization of the dendritic membrane and nerve impulses generated in the region of the receptor-cell soma.

Sensitivity of pheromone receptor cells and behavioural responses

Female moths are reported to attract their males via pheromones over distances of one km or more *(58, 59)*. While female insects may be unable to smell their pheromone, male insects often have enlarged antennae with numerous sensilla specialized for the most sensitive reception of the pheromone. For example male moths have α combed antenna which in extreme cases has an outline area of more than one cm^2, a size corresponding to one of the nostrils of a human nose. Interestingly these most sensitive insect antennae have an about tenfold smaller number of olfactory receptor cells than our nose, which contains a few millions of

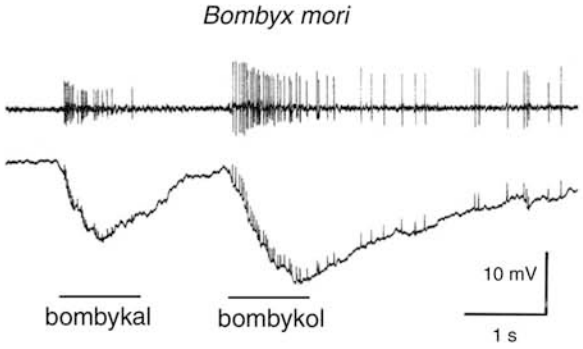

Fig. 8. Responses obtained from one hair with two receptor cells of a male moth of *Bombyx mori*. Upper trace AC-amplification, lower trace DC amplification showing receptor potentials with superimposed nerve impulses (spikes). One cell responds to bombykal (small spikes), the other to bombykol (large spikes) modified from (23).

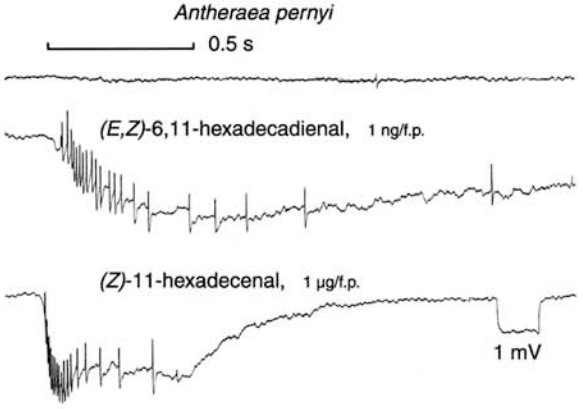

Fig. 9. Responses of a pheromone receptor cell of a male saturniid moth, DC-amplification. Upper trace: control response to air. Middle: response to 1 ng per odour source (filter paper) of the major pheromone component. Lower trace: Response to 1 µg/f.p. of the modified pheromone. Not only is the effectiveness of the stimulus reduced but also the time course of the response has changed.

cells. In Saturniid moths each antenna carries up to 100,000 sensilla trichodea with long hairs, each of them innervated by two or three pheromone receptor cells. The extreme sensitivity of moths to the pheromone - similar to that of a dog to certain odours - is due to the geometry and the arrangement of sensilla on the 'everted' insect antenna, which is optimized for capturing molecules from the air space (see below).

Combined radiometric, electrophysiological and behavioral studies *(4, 60)* were employed to study the absolute sensitivity of the silkmoth *Bombyx mori,* which will be briefly summarized here. An important tool was the radiolabeled pheromone bombykol *(61)*. Although a high specific activity was obtained by introducing one tritium atom per bombykol molecule the minimum amount detectable in a scintillation counter is at 10^{-8} bombykol molecules, or 4.10^{-8}µg of bombykol. The load of the stimulus source (1 cm^2 filter paper) at the 20% behavioral threshold of the male moths was 3.10^{-6} µg of bombykol (Fig. 10). The release of the odour source could be measured above 3.10^{-3} µg of bombykol, and the fraction of released stimulus molecules adsorbed on the antenna at even higher loads. The number of molecules per one of the 17,000 pheromone-sensitive hairs of the antenna was compared with the number of nerve impulses recorded from single olfactory hairs (sensilla trichodea), at loads at and above 10^{-5} µg. According to extrapolation, at the 20% behavioral threshold, each of the hairs received on average 0.04 molecules during the one-s stimulus and produced about 0.01 stimulus-induced nerve impulses. With stimulus loads between 10^{-5} µg and 10^{-3} µg, the number of nerve impulses elicited per stimulus increased linearly and obeyed a Poisson distribution for single random events, i. e. the arrival of single molecules. The final conclusion was that one pheromone molecule is sufficient to elicit a nerve impulse (spike) in a receptor cell. However, several hundred impulses per antenna need to be elicited in order to alert the moth, i.e., to overcome the noise of spontaneous firing of the receptor cells.

The comparison between spontaneous and stimulus-induced nerve impulse firing at the behavioural threshold shows that the moth CNS performs an extremely efficient

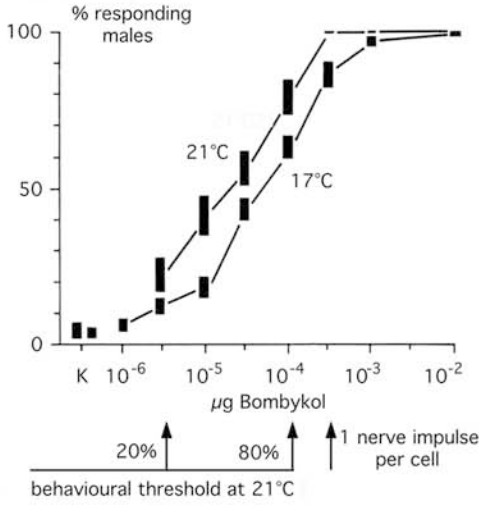

Fig. 10. Threshold curves of the wing vibration response of male *Bombyx mori* to 1-s stimuli at two room temperatures. Abscissa indicates the load of the odour source in µg per filter paper. For 21°C the 20% and 80% thresholds are indicated. The number of stimulus-induced nerve impulses per stimulus and per cell was 0.03 and 0.309 at 10^{-5} and 10^{-4} µg of bombykol per filter paper, with n=866 and 895 stimuli, respectively. Modified from (60)

signal-to-noise analysis as demonstrated by the following numbers taken from *(60)*. The nerve impulses elicited by a one-s stimulus of low intensity (10^{-4} µg of bombykol per odour source, eliciting 80% behavioural responses) occur during the first two s after stimulus onset, with an average delay of 510 ms. The start of the behavioural response (wing vibration) is also distributed over the first two s after stimulus onset. Its average delay exceeds that of the nerve impulses by about 200 ms, as measured at the 80% behavioural threshold and at up to 100-fold higher stimulus intensities. Thus the CNS integrates nerve impulses fired by the receptor cells for a time interval of maximally 200 ms until it elicits the behavioural response.

With 0.17 spontaneous spikes per cell within 2 s (from Table 2 in (60)) the average spontaneous activity of all 17,000 cells in one antenna is 291 spikes within the integration time of 200 ms. Since the spikes are about Poisson-distributed the noise of the spontaneous activity is the square root of 291 = 17 spikes. At the 80% behavioural threshold the average stimulus-induced signal from all cells within 200 ms is 525 spikes. Thus, the signal-to-noise ratio detected by the CNS is 525/17 = 31. At the 20% behavioural threshold (at $3x10^{-6}$ µg of bombykol per odour source) the signal-to-noise ratio would be 0.93, an amazingly low value.

Recently extremely sensitive cardiac responses to pheromone stimuli were reported for the moth *Spodoptera littoralis (62)* although the numbers of stimulus molecules released from the odour source and adsorbed on the antennae of this moth were not measured.

As in vertebrates olfactory receptor cells of insects are primary sense cells; they send their axons to glomeruli of the antennal lobe *(6)*. The pheromone receptor cells terminate in the macroglomerular complex (MGC) of the antennal lobe, which has a subunit for every type of pheromone receptor cell *(63)*. The convergence of primary fibres onto secondary neurons (local interneurons and projection neurons) in the MGC is about 1000 to 1. It must be here that the signal to noise analysis of pheromone-elicited nerve impulses takes place. There are functional connections between the MGC and the ordinary glomeruli which receive input from receptor cells for general odours *(64)*. The division of the antennal lobe into a region for the pheromone input and one for general odours *(42)* resembles that of the olfactory system in many vertebrates, where the accessory and the main olfactory bulb are innervated from the vomeronasal organ and the main olfactory epithelium, respectively.

The quantitative range of the receptor-cell response may cover several decadic steps of stimulus intensity. Often the nerve-impulse discharge is tonic at low and phasic at high stimulus intensities. After strong stimuli, the cells become less sensitive: they adapt and may need many minutes to recover. Sensory adaptation occurs at the level of the receptor potential and, even with fairly weak stimulus intensities, at the level of nerve-impulse generation *(65)*. Still weaker stimuli can cause habituation of behavioural responses due to adaptation processes within the CNS.

Olfactory transduction, extracellular

Transduction of an olfactory stimulus into a nervous response comprises extracellular and intracellular processes. Biophysical, biochemical, and electrophysiological studies suggest that extracellular processes may govern the kinetics of the first electrical response of the receptor cell, the receptor potential *(66)*. This implies that such events would proceed more slowly than intracellular signalling. Extracellular transducer processes include

- the adsorption of pheromone on the antenna, especially the olfactory hairs,
- the diffusion of the pheromone along the surface of the hairs towards the entrance of the pore tubules in the hair wall and crossing the hair wall along the pore tubules,
- the solubilization of the mostly lipophilic pheromone by binding to the pheromone-binding protein (PBP) in the sensillum lymph,
- the transport of the pheromone-PBP complex to the receptor-cell membrane,
- the activation of the receptor molecule in the plasma membrane ot the receptor cell,
- the deactivation of the pheromone and, finally,
- the enzymatic degradation of the pheromone.

An important tool for studying these processes was tritium-labelled pheromone. Using ^{3}H-pheromone the numbers of stimulus molecules released from the odour source and adsorbed on the antenna have been measured *(67)*. The large combed antennae of silkmoths adsorb about 30% of the pheromone molecules within the air passing over an area equal to the antennal outline. From the air making contact with the antenna itself all pheromone molecules are adsorbed. It can be calculated that a pheromone molecule - if it were reflected by the antennal surface - would hit the antenna about 100 times on its diffusional zig-zag path through the lattice of sensillar hairs on the antenna. Due to the spacing of the hairs tuned to the diffusional movements of the pheromone in air and due to the lipophilic surface of the hairs the antenna serves as an ideal sieve for catching molecules from the air space. Initially 80 % of the molecules adsorbed on the antenna were found on the hairs by measuring the radioactivity on hairs cut off immediately after stimulation with labelled pheromone. Thus the antenna serves as a kind of olfactory lens concentrating the molecules on the sensitive regions, the sensillar hairs with the receptor-

cell dendrites.

Radiolabelled pheromone was also used to determine the velocity of pheromone transport to the receptor cells. Since the hairs comprise a relatively small volume of the antenna, the initial concentration of pheromone adsorbed is very high. Following the concentration gradient the molecules migrate from the hairs to the antennal body. By cutting hairs of male antennae at various time intervals after stimulation with labelled pheromone, the velocity of this migration was determined. The coefficient for longitudinal diffusion was 5 x 10^{-7} cm^2/s for ^{3}H-bombykol ((E,Z)-10,12-hexadecadienol) in *Bombyx mori (68)* and 3 x 10^{-7} cm^2/s for ^{3}H-(E,Z)-6,11-hexadecadienyl acetate, the major pheromone component of the saturniid moth, Antheraea polyphemus (67). This velocity corresponds to the range expected for diffusion within the sensillum lymph of the PBP molecule with a MM of 15 kD. Since the radioactivity was shown to enter the sensillum lymph it was concluded that the longitudinal diffusion occurs while the pheromone is bound to the PBP.

A fast migration of the pheromone molecule from the adsorption site on the hair surface to the pores of the hair wall and to the inner end of the pore tubules is expected from the distance between pores (0.5 µm), the thickness of the hair wall (below 0.5 µm), and the velocity of migration on the cuticular hair wall. The latter velocity was determined from the longitudinal migration along hairs of dried antennae *(67)*. The sensillum lymph was evaporated, - and the hairs filled with air, so that the PBP was unable to diffuse. On these hairs the longitudinal migration was about 3-fold faster than in intact antennae. With this velocity the expected mean time between adsorption on the hair surface and arrival at the inner end of the pore tubule is in the ms-range. A further delay of about one ms must be ascribed to the diffusion of the pheromone-PBP complex from the inner end of the pore tubule to the receptor-cell membrane over a distance of about 1 µm. Interestingly, the total estimate of the average delay between pheromone adsorption and its arrival at the cell membrane is less than 1 % of the average delay of a nerve impulse (510 ms, see above). Quantitative modeling of the perireceptor events suggests that the delay is mainly due to the speed of pheromone-receptor association and of pheromone deactivation *(66)*. At low stimulus intensities the delay of nerve impulses has a broad distribution *(60)*. At strong stimulus intensities the receptor potential starts after a few (below 10) ms and the first nerve impulse is elicited after another few ms.

Pheromone deactivation and enzymatic pheromone degradation

At physiological stimulus intensities there is practically no desorption of the pheromone from the antenna *(67)*. This means that the amount of pheromone taken up by the antenna increases during exposure to pheromone and stays constant after stimulus offset. However, the electrophysiological response does not increase indefinitely during constant stimulation, but rather levels out and starts to decline immediately after stimulus offset. Therefore a process was postulated which keeps the concentration of active stimulus molecules on the antenna constant by deactivating the stimulus molecules shortly after uptake *(69)*. From the decline of the receptor potential after stimulus offset and considering the dose-response relationship of the receptor potential amplitude a half life of 0.75 s can be estimated for the active pheromone adsorbed (calculated from kfall = 0.924/s of *(66)*). The process of deactivation seems to be saturable since after extremely strong stimuli the response does not decline but continues for a time interval depending on the stimulus strength. In order to account for its saturation the deactivation was modeled as a process catalyzed by a hypothetical enzyme N *(66)*. Alternatively it was assumed that the receptor molecules themselves could act as enzymes catalyzing deactivation *(70, 71)*.

Enzymatic degradation of pheromone was indeed found on living antennae, initially by Kasang *(72)* for bombykol in the silkmoth *Bombyx mori*. Living antennae exposed in air for 10 s to ^{3}H-bombykol were subsequently eluted for 10 min by pentane and for another 10 min by a chloroform-methanol mixture, and the amounts of bombykol and its metabolites in the resulting solutions were checked by thin-layer chromatography. When elution was started three min after exposure, 50% of the bombykol had been turned into aldehyde and acid. Later elutions also included esters. The degradation was sensitive to temperature, suggesting catalysis by an enzyme, probably a dehydrogenase. Interestingly, pheromone degradation was also found in female Bombyx antennae lacking pheromone receptor cells, and on other body parts such as the wings or legs of both sexes *(73 - 76)*. Since these body parts are tightly covered with scales, the pheromone degradation also occurs on these cuticular structures devoid of cellular elements. Vogt and Riddiford *(77)* isolated an enzyme from body scales, an interesting case of enzymatic reactions in non-aqueous material.

From the halflife of intact pheromone of three min, it follows that the pheromone degradation on living antennae is about 200-fold too slow to account for the decline of the receptor potential after stimulus offset. Therefore a more rapid deactivation was postulated, by a process that leaves the pheromone chemically intact. Enzymatic degradation may nevertheless also have a useful function, in removing traces of pheromone left over from incomplete deactivation *(66)*. This is important to guarantee full recovery of the receptor cells from previous stimulation and to reduce the nerve

impulse discharge to the level of spontaneous activity. The degradation on the entire body surface prevents the generation of secondary pheromone sources that could interfere with the mating behaviour of the males.

Pheromone-degrading enzymes (esterases, aldehyde oxidases) were isolated from moth antennae *(78, 79)*, and cloned (80). Also enzymes belonging to the cytochrome P450 family were found in moth antennae *(81)*. Analysis of droplets of sensillum lymph collected from cut hairs showed that pheromone degrading esterase is present inside the hairs (82). The investigation of enriched esterase revealed a Km of 2.2 µM, a catalytic rate constant in the range of 30/s, and an estimated concentration *in vivo* of 1 µM *(78)*. These values imply that pheromone degradation in vivo should be much faster (16 ms half life) than actually found in living antennae (3 min half life, see above). The discrepancy between the expected value and the in vivo measurement can be resolved by considering the finding of Vogt and Riddiford *(83)* that the speed of degradation is reduced in the presence of pheromone binding protein (PBP) *(66)*.

That the degradation is not responsible for the decline of the receptor potential is supported by the lack of correlation between that decline and the degree of enzyme activity in single antennae *(84)*. The idea of two processes, a faster deactivation followed by a slower degradation is also supported by the electrophysiological response to the bombykol derivative (Z,E)-4,6-hexadecadiene *(85)*. A one-s stimulus by this compound produces a response, which - after stimulus offset - declines like a response to bombykol; in addition it produces a long-lasting (15 min) firing of nerve impulses of the bombykol receptor cell. The initial decline could be due to deactivation whereas the post-stimulatory firing may indicate that the hexadecadiene cannot be degraded by the dehydrogenase postulated by Kasang (see above).

The enzymatic pheromone degradation found by Kasang on living antennae *(72)* is incorporated in the model of Kaissling *(66)*. The pheromone bound to the scavenger (called Box in the model) must be accessible to the enzyme although e.g. with a 1000-fold smaller rate constant for the association of pheromone and enzyme compared with the association with the free enzyme (rate constant k10 compared with k8). This factor 1000 represents the 'protection' from enzymatic degradation of the pheromone by binding to the PBP *(83)* and is used in the model to get the 3-min half life of bombykol (see above). So the protection is not absolute - in accordance to the protection experiment *(83)*.

The mechanism of pheromone deactivation is unknown. It is possible that a separate protein acts as scavenger but it seems also likely that the PBP is involved *(66)*. The PBP could become 'locked', i. e. irreversibly bind the pheromone and thus make it unavailable to the receptors. This was suggested by the finding of the 'redox shift' of a PBP of *Antheraea polyphemus (70)*. Experiments with reducing agents (1,4-dithio-DL-threitol) and splitting at SH-groups (using 2-nitro-5-thiocyanobenzoic acid cleavage) suggested that the major PBP of this moth can adopt two forms, which migrate as separate bands with different velocities in native polyacrylamide gels. The faster migrating, oxidized form has three intramolecular disulfide bridges, whereas the more slowly migrating, reduced form has one or two disulfide bridges. In the presence of the pheromone and of an unknown factor within hair homogenates the more abundant reduced form turned into the oxidized form. The unknown factor may be the hypothetical enzyme N. This redox shift measured *in vitro* gave rise to the following working hypothesis. The reduced form acts as a carrier of the lipophilic pheromone through the watery sensillum lymph. The reduced PBP-pheromone complex activates specific receptor molecules in the dendritic cell membrane and induces the cell response. The oxidized form encloses the bound pheromone and prevents it from activating further receptors, thus acting as a scavenger *(70)*. The in vitro velocity of this process was about tenfold smaller than expected from the decline of the receptor potential, probably due to partial deterioration of the preparation. The existence of the two PBP forms was not confirmed by mass spectroscopy; the expected difference of reduced and oxidized form by two H atoms was not observed *(86)*. This suggested that the two bands of the *A. polyphemus* PBP may instead be conformers of the same (oxidized) molecule. The conflicting evidence needs experimental clarification.

The existence of the postulated pheromone deactivation would mean that an extracellular process governs the kinetics of the receptor potential. This conclusion seems inevitable since small alterations of the pheromone molecule not only reduce the response amplitude but also change its kinetics. For instance stimulation by several (less effective) pheromone derivatives leads to a faster rise and decline of the receptor potential than observed with the pheromone itself *(66, 71)* (Fig.9). Thus, a compound must appear to be less effective if it is more quickly deactivated. One should generally take into account that processes such as the postulated pheromone deactivation or the binding of the odorant to extracellular binding proteins (see below) may contribute to the response specificity *(87)*. However the receptor-cell specificity seems mainly bound to the cell, and is most likely determined by the interaction of stimulus molecules with receptor molecules since the specificity of binding to PBPs seems less sharp than the specificity of the cell response (see below).

Adsorption without desorption, followed by deactivation constitutes a flux detector system *(71, 88)*. For a flux detector the antennal uptake of stimulus molecules does not only

depend on the stimulus concentration in air but also on the airstream velocity relative to the antenna. Multiplication of concentration by velocity gives a flux value in molecules per cm^2 per s. In concentration detectors, the receptor molecules are directly exposed to the stimulus concentration, and an equilibrium between adsorption and desorption is rapidly established during stimulation so that the airstream velocity has no noticeable effect on the stimulus uptake. Concentration and flux detectors differ with respect to the dose-response relationships between stimulus intensity and occupation of the receptor molecules by the stimulus molecule *(71)*. Whereas the relationship is hyperbolic in concentration detectors it can be quasi-hyperbolic, linear or steeper than linear in flux detectors depending on whether the binding to the receptors is stronger, equal to or weaker than the enzymatic reaction, respectively. Taste organs might be concentration detectors, but most olfactory organs are likely to be flux detectors. One exception may be insect receptors for atmospheric carbon dioxide which do not respond to the airstream velocity.

Functions of pheromone binding proteins

PBPs were first described by Vogt and Riddiford (89). A typical PBP has a MM of 15 kD and 142 amino acids, and possesses six highly conserved cysteines forming three disulfide bridges *(90 - 92)*. The amino acid sequence is known for many of these proteins. Species with a larger number of pheromone components possess a diversity of PBPs with different binding specificities *(93)*. The concentration of the PBP within the sensillum lymph is extremely high, in the range of 10 mM *(78, 82)* (Fig. 6C). Several species of homologous PBP molecules may occur in the same sensillum *(94)*. Non-pheromone sensilla contain so-called general odorant binding proteins (GOBPs), which are related to the PBPs and share the six conserved cysteines *(9, 87, 95)*. Besides these odorant binding proteins (OBPs) further proteins of lower homology with possible chemosensory function (chemosensory proteins, CSPs) have been found in several insect orders *(23, 97, 98)*. Drosophila melanogaster has about 40 PBP-related proteins *(99)*. It remains to be shown whether all of these proteins are functional.

PBPs seem to serve multiple functions. In summary, the PBP

1 binds the pheromone,
   - a water-solubilizes the lipophilic pheromone,
   - b transports the pheromone through the sensillum lymph (see above),
   - c contributes to the specificity of the receptor-cell response,
   - d prevents the pheromone from integration into the cell membrane (suggested by unpubl. experiments with pheromone and liposomes),

2 protects the pheromone from enzymatic degradation (see above),

3 may be involved in pheromone deactivation (suggested by the redox shift of the PBP, see above),

4 is involved in the interaction of pheromone and receptor molecule (53),

5 binds and removes non-pheromonal compounds (hypothetical),

6 provides organic anions to the sensillum lymph.

Re 1) The PBP was detected using gel electrophoresis of PBP with ^{3}H-labelled (E,Z)-6,11-hexadecadienyl acetate, the main pheromone component of *Antheraea polyphemus (89)*. The binding survives the electrophoresis. The first binding assay took advantage of the strong binding of this pheromone to a glass surface. Adding PBP to the buffer solution with ^{3}H-pheromone within the glass vial solubilized the pheromone until one pheromone per PBP molecule was bound *(100)*. This assay revealed a dissociation constant of 60 nM. A weaker binding (Kd = 640 nM) was found by a different assay for the same PBP preparation by Du et al. *(101)*. The solubilization was also shown during electrophysiological recording by direct application of the polyphemus pheromone and the PBP to the sensillum lymph via the recording glass capillary (superfusion) *(52)*. A similar solubilization was also obtained using bovine serum albumin (BSA) instead of PBP. The pheromone-PBP complex seems to migrate within the hair lumen along the hair (see above). Finally the radioactivity was found in the hemolymph of the antenna (unpubl. observations).

According to the binding assays and also competition assays, the pheromone-PBP binding has some specificity, but it is weaker and often different from that of the cell response. Nevertheless it may contribute to the specificity of the latter. Thus in *Antheraea polyphemus* (E,Z)-6,11-hexadecadienol was 1000-fold less effective as a stimulus than the pheromone (E,Z)-6,11-hexadecadienyl acetate and bound 1000-fold less to the PBP *(54, 101, 102)*. However, binding of the saturated acetate to the isolated PBP was only 10-fold weaker, whereas its effect on the cell response was one million times weaker than that of the pheromone. The dissociation constants of (+)- and (-)-disparlure and two recombinant PBPs in the gypsy moth differed by about 2- to 4-fold *(103)*. In contrast, the sensitivities of both types of receptor cells for the two enantiomers differed by factors of more than one hundred *(104)*.

The same sensillum may contain several PBPs with different binding specificity . Thus there are three types of PBPs in the sensilla trichodea of *Antheraea polyphemus* and *A. pernyi*, together with three receptor cells, each tuned to one of the three pheromone components *(94)*. Each of the PBPs binds preferentially one of the pheromone components. This is in agreement with the observation of Mohanty et al. *(105)* that certain amino acids in the pheromone binding cavity (see below) play a role in chain length recognition by the PBP. The various types of PBPs occur in very different amounts *(94)* and the binding constants to their preferred pheromone components are unknown (except for the main PBP of *A. polyphemus*, see above).

Re 4). Recent observations in silkmoths indicate that PBPs are probably also involved in the odorant-receptor interaction *(53)*. The sensillum lymph bathing the odor-sensitive processes of the receptor cells can be partially exchanged while the cells are functional (superfusion *in situ*). When the receptor cells were superfused with a combination of a PBP 'X' and a non-preferred pheromone component 'Y', a response was elicited from the cell 'Y' tuned to the pheromone 'Y' that was actually present. But also the second cell 'X' fired that was tuned to the (absent) pheromone component 'X' preferred by the PBP 'X'. Since the latter component 'X' was not present the PBP 'X' itself must have somehow participated in the excitation of the second cell. Actually the PBP here impaired the selectivity of the cell response. In some cases even the free PBP of a different moth species elicited a response, without a pheromone added.

Ad 6). Dispersive X-ray elementary analysis of sensillum lymph microdroplets revealed a lack of anions *(43, 44);* only about half of the anions are covered by chloride. This is compensated by the PBP with an isoelectric point (pI) of 4.5 and its high concentration range of 10 mM *(82)*. For instance the PBP of *Bombyx mori* at neutral pH possesses 23 negative and 14 positive charges. Incidentally, the analysis also revealed a high sulfur peak which may be ascribed to the sulfur content of the PBP (e.g., 12 sulfur atoms per molecule in Bombyx mori).

Finally it should be noted that PBP-related proteins with unknown function also occur in insect taste receptors *(106, 107)*.

Structure of the PBP molecule

Recently the tertiary structure of the bombykol binding protein was analysed by X-ray crystallography *(108)* and by NMR *(109)*. The PBP consists of a chain in which hydrophilic and hydrophobic amino acids alternate, the whole chain forming six or seven alpha-helices folded up into an apparently tangled ball. The helices are connected to one another by three disulfide bridges between six highly conserved cysteines. Most of the hydrophilic amino-acid residues face outward and most of the hydrophobic ones, inward (Fig. 11). The predominantly hydrophilic outer surface makes the protein water-soluble, while the interior of the ball forms a central cavity with a hydrophobic lining, which can accommodate the odor molecule. The nature of the bombykol-PBP interactions was studied by *(110)*.

Interestingly, this principle of a double-walled nanocapsule has been implemented at least twice in evolution. Odorant binding proteins of mammals belong to the lipocalin family whith a similar size and function as the insect OBPs, also called encapsulins *(86)*. However lipocalins, serving as odorant binding proteins in vertebrates *(14)*, have a different structure characterized by antiparallel beta-sheet folding and in addition comprise two alpha helices near the N terminal. The sheets held together by hydrogen bridges, form a container-like structure called the 'beta barrel'.

The structural analysis of the insect PBP indicated still more of its functions. A particularly exciting finding is that a PBP may alter its binding capability for the ligand. In the silkmoth Bombyx mori the binding of the pheromone bombykol was found to be disrupted at low pH. Structural analysis of the bombykol-binding protein by X-ray and NMR provided the explanation: the PBP undergoes a spectacular pH-induced conformational change. X-ray crystallography showed that the bombykol molecule is enclosed in the interior cavity of the PBP while the latter is in its neutral or basic B-form (Figs. 11, 12). When the PBP is examined in a

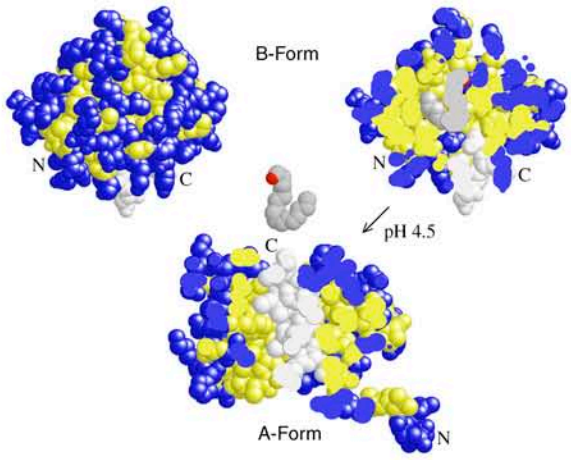

Fig. 11. Structure with hydrophilic amino acid residues in blue and hydrophobic residues in yellow. B-form (above, complete and half-cut) from X-ray analysis (108). A-form (below, half-cut) at pH 4.5 from NMR analysis (109). The B-form binds bombykol (white, with red OH-group) in an inner cavity. The A-form does not bind the ligand. Its inner cavity is occupied by the C-terminus. (Made by RasMol)

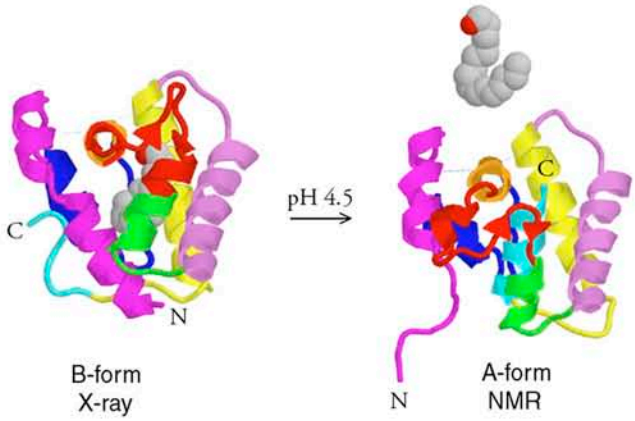

Fig. 12. Backbone of the Bombykol binding protein showing the pH-dependent conformational changes. At low pH the C-terminus forms a new helix entering the internal cavity, the loop (in red) between helix 3 and 4 flips by 180°, and the N-terminus partially unfolds. (Made by RasMol)

solution at neutral pH by nuclear magnetic resonance (NMR), it can be seen that the cavity remains open even without the bombykol present *(111)*. However, in the acidic pH range (pH 4.5) the binding capability is lost, and the secondary and tertiary structure of the protein (A-form) are completely changed. As a result of the pH reduction, the five histidine residues of the protein are protonized, and electrostatic effects drive the protein components apart and thus expand the interior cavity of the protein. Three major changes occur (Figs. 11, 12): The predominantly hydrophobic C terminus of the PBP forms a new helix and enters the cavity, filling it completely so that there is no longer any room for the ligand. In addition, the loop between helices three and four bends away by about 180°, opening a lid for the release of the bombykol *(112)*. Finally, the predominantly hydrophilic N-terminal helix unfolds.

Model experiments with artificial lipid membranes make it seem likely that the conformational change described can occur when the PBP molecule approaches the cell membrane to within a few Angstroms. The change might well be initiated when protonation of the histidine is induced by the low pH locally generated due to negative charges attached to the cell membrane *(113)*.

These observations suggest that the conformational B⇒A change functions to release the ligand from the internal binding cavity, and thus enables the pheromone to interact with the receptor molecule of the cell membrane. The pheromone is 'presented' to the receptor. Probably the conformational change occurs not only at the cell membrane but also at the inner hair wall helping the incoming pheromone to enter the binding cavity. This is suggested by the finding of negative charges attached to the fine tubules through which the odorant molecules cross the hair wall *(114)* (Fig. 13). Thus the incoming pheromone molecules could attach to the A-form and - as a result of the A⇒B change - be swallowed into the inner binding cavity of the ferry. This way the pheromone is solubilized and can be transported towards the receptor cell membrane (Fig. 14).

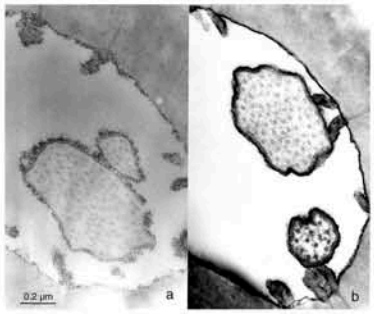

Fig. 13. Cross-sections of olfactory hairs of male *Antheraea polyphemus* treated with cationic markers, demonstrating that negative charges are associated with the dendritic membrane but also with the inner ends of the pore tubules and the inner surface of the hair wall (114).

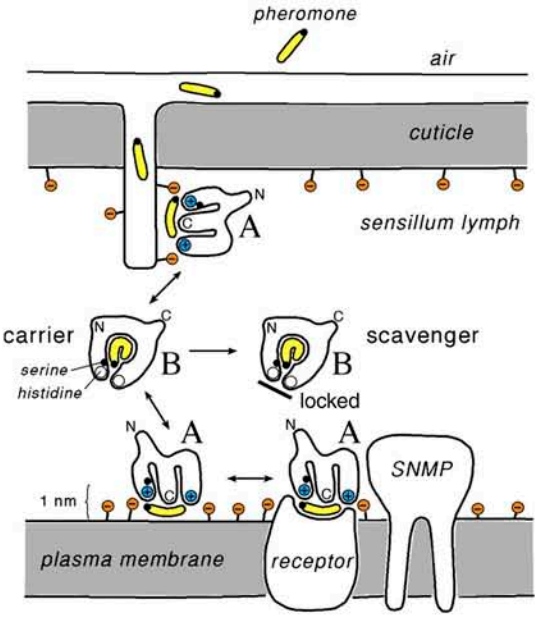

Fig. 14. Scheme of possible perireceptor events in the hair lumen. The PBP approaches a region of negative charges at the pore tubules, adopts the A-form, takes up the lipophilic pheromone, changes to the B-form and transports the pheromone within the internal cavity through the aqueous sensillum lymph, adopts the A-form due to negative charges at the receptor cell membrane and releases the pheromone for interaction with the receptor molecules. Finally the odorant has to be deactivated, possibly by a locking process which prevents a further release of the pheromone from the PBP. The membrane-associated SNMP might assist docking of the PBP to the cell membrane.

The tight encapsulation of the pheromone shown by X-ray analysis supports the idea of a mechanism of deactivation by imprisoning the pheromone in the interior of an OBP (Fig. 14). This would require a special process which blocks any further B⇒A change, e. g. the hypothetical enzymatic deactivation process discussed by *(66)*.

Since the structure of the *Bombyx* PBP (BmorPBP) became known, a few other antennal PBPs of insects have been analysed. The ApolPBP1 of the moth *Antheraea polyphemus* is similar in secondary and tertiary structure to BmorPBP, form B *(105)*. ApolPBP1 has 5 histidins, its acidic structure is not yet known. The PBPs of the cockroach *Leucophaea madera* (LmadPBP, binding a pheromone component, *(115)*), the honey bee *Apis mellifera* (Amel-ASP1, binding two major pheromone components *(116)*), and the fly Drosophila melanogaster (LUSH, binding short-chain n-alcohols *(117)*) show interesting differences to the bombykol binding protein, e. g. only two histidines in LUSH, one in Amel-ASP1, and none in LmadPBP. The C-terminus of Amel-ASP1 is placed tightly to the 'body' of the protein, along the wall of the internal cavity. LmadPBP has a C-terminus shortened by 24 amino acids. Thus pH-dependent changes such as found in BmorPBP are not expected for all PBPs. Other chemosensory proteins (CSPs) with as yet unknown functions have also been found in insects *(13)*. These are similar in size to or smaller than OBPs , but differ in amino acid sequence. They have less than 6 cysteines and seem more flexible and less selective regarding their ligands.

Of particular interest is a membrane protein called SNMP (sensory neuron membrane protein), a member of the so-called CD36 protein family *(118 - 120)*. This family of cell development proteins is also represented in vertebrates, for instance by a protein in mammalian milk. Its members are characterized by two terminal transmembrane domains and a large extracellular domain; they function as docking sites, where extracellular protein molecules can become coupled to the cell membrane. The SNMP of *Antheraea polyphemus* is present in high density in the olfactory cell membrane (Fig. 6A,B) and could help in docking the pheromone-PBP complex at the receptor molecule (Fig. 14).

Olfactory receptor molecules

Insect pheromone receptor molecules still await identification. The high selectivity of pheromone receptor cells suggests that each cell type comprises a single type of receptor protein, expressed from one gene. In *Drosophila melanogaster* more than 60 different candidate odorant receptor molecules have been identified, each having seven transmembrane domains activating G-proteins. Each receptor cell expresses only one type of receptor protein *(121, 122)*. Recently, molecules belonging to the seven-transmembrane-domain category were also identified and localized by in situ hybridization in antennae of the moth *Heliothis virescens (123, 124)*.

In spite of their hypothetical nature, binding properties and number of pheromone receptor molecules have been investigated by indirect approaches based on electrophysiological and biochemical data. Extracellular recordings from single sensilla under "loose patch" conditions with very weak stimulus intensities showed transient receptor potentials or currents. They appear as a single "bump" of 10ms duration, or bursts of a few bumps, preceding a single nerve impulse, seldom two or more nerve impulses. They also may occur without being followed by a nerve impulse. These "elementary" responses can be elicited by single pheromone molecules, probably interacting with a single receptor molecule and may reflect the pattern of its activation *(125)*. They could reflect transitions among three states of the pheromone receptor molecule: the vacant receptor (state 1), the pheromone-receptor complex (state 2) determining the burst duration, and the activated complex (state 3) producing a bump. The analysis of the duration of bumps and the gaps between bumps within a burst, as well as the burst duration's and the numbers of bumps per burst, revealed rate constants of the transitions between states in a three-state model: k_{21} = 7.7/s, k_{23} = 16.8/s, and k_{32} = 98/s.

By quantitative modeling *(66)* the density of receptor molecules was estimated as at least 3,000 per μm^2 of the receptor cell membrane for *Bombyx mori*. This corresponds to >7.6 % of the density of rhodopsin molecules in the disc membrane of visual receptor cells (40,000/μm^2). In the moth *Antheraea polyphemus* the corresponding relative density was >15%.

The calculated (minimum) value for the dissociation constant of the pheromone-receptor complex is unexpectedly high (K_d = 35.4 µM). This demonstrates that the high sensitivity and selectivity of the cell response does not require a high affinity between pheromone and receptor molecule. Interestingly, the affinity of pheromone and receptor molecule is much weaker than that of the pheromone and the extracellular PBP, with K_d values as low as 60 nM.

Olfactory transduction, cellular

According to an analysis of the electrical equivalent circuit of the sensillum, the bumps are produced by an average increase of dendritic membrane current in the range of 1.5 pA, by opening either a single ion channel or several channels with smaller current per channel *(56)*. The number of active channels cannot be determined in transepithelial recordings due to the capacitances of the sensillum circuit. Openings of ion channels, 56 pS each, with mean opening durations of 1.2

ms and a mean open probability of 0.24 were observed in cell-attached patch clamp recordings from pheromone receptor cells of the moth *Antheraea polyphemus (126)*. Simulations reveal that openings of three or four such channels could produce an elementary receptor potential *(125)*. Activation of the receptor molecule probably leads to a transient release of second messenger, which opens a few channels in the neighbourhood of the release site.

After the activation of receptor molecules by the odorant, a variety of intracellular signal compounds seems to be involved in the transduction process; among them are 1,4,5 inositol trisphosphate, diacyl glycerol, cGMP, and Ca^{++} (127, 128). While various constituents of pathways have been identified and immunolocalized (129 - 131) signalling is not yet understood at a quantitative level (132, 133). In dendrites of the moth Antheraea polyphemus channel openings (56 pS) were observed upon stimulation of inside-out patches with cGMP (1µM) or diacylglycerol (=0.36 µM), in the presence of MgATP, but not by IP3 (1µM) *(126, 139)*. It has not been excluded that ion channels may be gated directly by the pheromone-receptor interaction.

According to electrical circuit analysis the resting membrane potential of the receptor cells and the transepithelial potential are maintained by conventional Na/K pumps in the soma region of the receptor cells together with an electrogenic potassium pump in the distal membrane of the auxiliary cells *(43)*. The latter pump is responsible for the high potassium concentration in the sensillum lymph. More than one type of ion channels appears to contribute to the receptor potential, and further channels must be involved in the generation of nerve impulses in the soma region of the receptor cell *(127)*. In *Antheraea polyphemus* the initial burst of nerve impulses observed at relatively high stimulus intensities might be induced by opening of a Ca^{++}-activated non-specific ion (CAN) channel located in the soma region of the receptor cell *(134)*. This phasic response adapts very quickly possibly because this type of channel is blocked by cGMP. The cloned cDNA of a cyclic nucleotide and voltage-activated ion channel from the antennae of the moth *Heliothis virescens* was heterologously expressed and analysed by patch clamp recordings and in situ hybridization *(135)*. It was suggested that this channel plays a role in regulating the responsiveness of the cell via intracellular cAMP-levels, possibly controlled by the neuromodulator octopamine *(136, 137)*.

Inhibition of pheromone receptor cells

Inhibition has also been observed in pheromone receptor cells, but it is not known whether this has a biological function. For instance, receptor potentials and the nerve impulse responses can be completely and reversibly abolished by terpenes, geraniol in *Antheraea polyphemus (138)* or linalool in *Bombyx mori (85, 139)*. Pulses of linalool stimuli given at a rate of 3/s have been used to modulate long-lasting poststimulatory impulse firing caused by (Z,E)-4,6-hexadecadiene *(85)*, and thereby to induce anemotactic walk of Bombyx males *(140)*. Clearly these terpenes are structurally not related to the pheromone compounds whose excitatory action they inhibit. However, they are not general inhibitors of olfactory receptor cells because other olfactory receptor cells, even in the same species, are excited or not affected by these compounds.

Recently, an instant inhibition of responses to pheromone was observed following exposure to decanoyl-thio-trifluoropropanone (DTFP) (Fig. 15), a volatile inhibitor of the sensillar esterase, the enzyme, that degrades a pheromone component of *Antheraea polyphemus (141)*. If applied at high concentrations, this compound produces a rapid repolarization of the transepithelial potential (Fig. 16) similar to that produced by general anaesthetics. DTFP inhibited pheromone receptor cells in various moth species. However, it did not inhibit non-pheromone cells. This compound binds very strongly to all PBPs tested *(94)*. The number of 3H-labeled DTFP molecules adsorbed per antenna necessary for inhibition was less than 1 % of the number of PBP molecules *(142)*. It was, however, about equal to the maximum possible number of receptor molecules on the receptor-cell membrane. In conclusion, these observations indicate that DTFP inhibits - probably while attached to the PBP - by occupying the site on the receptor molecule at which the pheromone molecule is recognized. DTFP and other trifluoromethyl ketones interfere with behavioural responses to pheromone and may be used in insect pest control *(143, 144)*.

There are compounds which more generally inhibit but also irritate the cells if applied at high concentrations, including many amines *(69, 145)*. They inhibit pheromone receptor cells as well as other types of receptor cells. Often they inhibit the cell at lower and excite it at higher concentrations. Such compounds might interfere with the lipid structure of the membrane so as to reduce membrane conductance at low doses. At high doses they cause increased conductance, probably destabilizing the membrane. After such stimuli recovery can be incomplete indicating irreversible damage of the cell function. There are compounds which excite and inhibit at the same time. Often the inhibitory effect disappears more quickly than the excitatory one, leading to poststimulatory rebound effects *(139, 146)* like those observed after simultaneous exposure to pheromone and general anaesthetics *(147)*.

When applied alone, these general anaesthetics cause hyperpolarization and suppression of spontaneous impulse

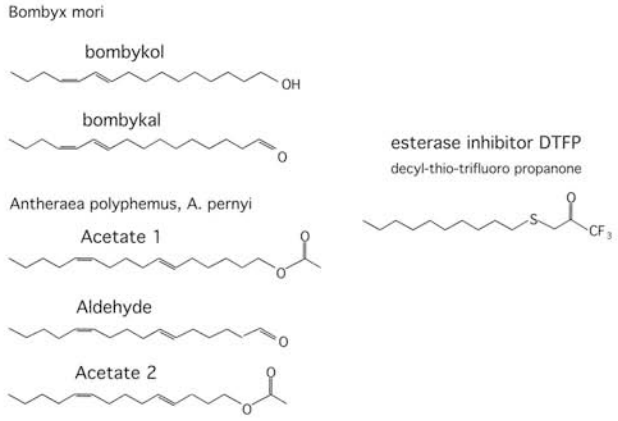

Fig. 15. Structures of moth pheromones and of the inhibitor DTFP, which (probably while bound to PBP) occupies the receptor sites for pheromone.

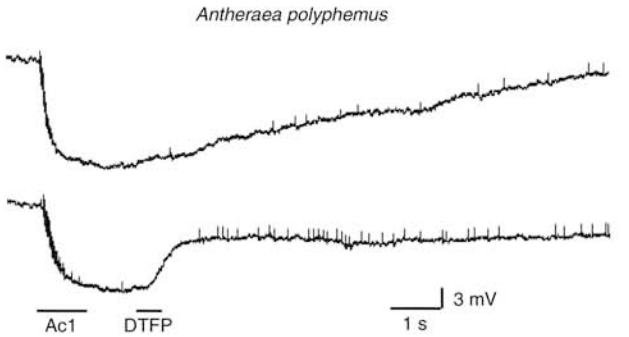

Fig. 16. Immediate blocking effect of DTFP on the receptor potential induced by pheromone stimulation. Two responses of a single receptor cell to the pheromone component Ac1 (see Fig 15). An initial burst of nerve impulses superimposed on the receptor potential can be seen during the increase (downwards) of the latter. Spiking ceases while the receptor potential levels out during stimulation. The DTFP stimulus (bottom trace) causes an immediate repolarization of the potential. This is followed by a recovery of nerve impulse firing, which is faster than after the uninhibited response. Modified from (141)

firing *(147)*. They also block the responses to pheromones or other key compounds. If applied during or directly after an excitatory stimulus, they rapidly repolarize the cell. Using local stimulation of the long sensilla trichodea, it has been shown that general anaesthetics do not block the response to pheromone unless they are applied at the same locus as the pheromone. Thus, they might impair the function of receptor molecules or ion channels in the receptor cell membrane either directly, or indirectly by interfering with the structure of the surrounding lipid matrix. Insecticides such as (+)-trans-Permethrin and DDT blocked the nerve impulses but not the receptor potential *(148)*.

Temporal coding

With stronger stimulation, the elementary receptor potentials add up to an overall receptor potential that can reach -30 mV. While the average latency of the elementary responses is about 0.5 s, at high stimulus intensities the onset of the overall receptor potential may be delayed by 10 ms only *(66)*. At high stimulus intensities, insect olfactory receptor cells and also higher-order neurons within the antennal lobe *(149)* resolve repetitive stimulus pulses up to frequencies of 10 pulses per s *(150 - 152)*. The time resolution measured depends on the type of receptor cell, - and on temperature (Fig. 17). The astonishing resolution is restricted to higher stimulus intensities where the response latency is short, with a minimum of about 10 ms. It was first shown by Kramer *(153)* that the anemotactic approach of an insect near the odour source consists of several turns per second into the upwind direction *(47, 154, 155)*. Each turn is elicited by a brief odour pulse caused by encountering the pheromone-containing air filament that originates from the female gland (Fig. 18).

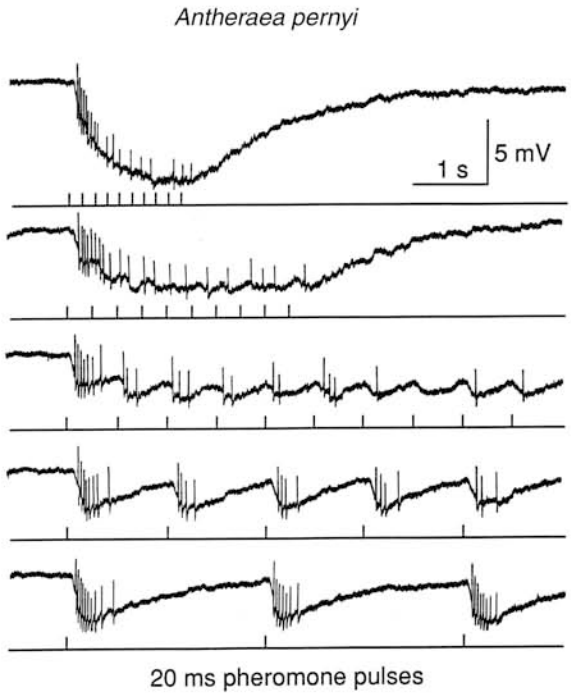

Fig. 17. Responses of a single pheromone receptor cell repetitively stimulated at various frequencies by pheromone pulses of 20 ms duration, at 8°C . Modified from (152)

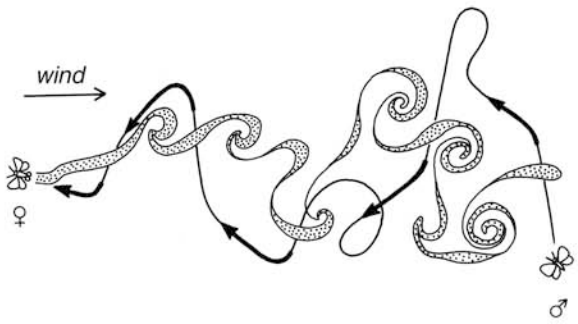

Fig. 18. A male moth flying within a turbulent odour plume released by a female moth encounters intermittent pheromone pulses. The male of *Bombyx mori* reacts with upwind turns (arrows) upon each stimulus pulse up to three pulses per s. Schematic diagram after (153).

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