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The Sensitivity of the Insect Nose: The Example of *Bombyx Mori*

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Abstract. The male silkmoth *Bombyx mori* responds behaviourally to bombykol concentrations in air of 3,000 molecules per ml presented at an air speed of 57 cm/s, i.e. the moth is almost as sensitive as a dog. The number of bombykol receptor neurons per antenna is 17,000, about 10,000-fold smaller than olfactory neurons found in dog noses. This high sensitivity is possible due to a very effective capture of odorant molecules and transport to the receptor neurons. The effectiveness of the insect antenna/nose has been determined by using radiolabeled bombykol, counting nerve impulses generated by the receptor neuron, and measuring the behavioural response of the male moth. At the behavioural threshold the neuronal signal/noise discrimination works at the theoretical limit.

3.1 Introduction

For a low olfactory threshold several sensory functions need to be optimized. Odour molecules have to be a) effectively caught by the antenna from the air space, and b) conducted with little loss to the olfactory receptor neurons. c) The odour stimulus has to be most sensitively transduced into nerve impulses, and d) the stimulus-induced impulse firing has to be distinguished from the background of spontaneous impulse discharge from the unstimulated receptor neurons. This paper reviews quantitative work on these items in the male moth of a species which is attracted (i.e. stimulated to walk upwind, Kaissling 1997) by a single chemical pheromone component, (E,Z)-10, 12-hexadecadienol (bombykol) released by the female moth (Butenandt et al. 1959).

3.2 Molecule Capture by the Antenna

To investigate the effectiveness of molecule capture by the antenna we used ^{3}H-labelled bombykol (Kasang 1968; Schneider et al 1968). With a high specific activity of 31.7 Ci/g, or one ^{3}H-atom per four bombykol molecules, about 10^{9} molecules or $4x10^{-13}$ g were required for a measurement in the scintillation counter. The odour source, a 1cm^{2} piece of filter paper (f.p.), had to be loaded with $3x10^{-12}$ g of bombykol in order to induce wing fluttering of some of the moth with a ten-s stimulus. Almost all of the responses occurred within two s. The threshold curve (in % of moths responding within the first two s) covered about 2 decades of stimulus

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load. Depending on temperature, the time of the day, and the animal origin the 50% threshold was reached at loads between 10^{-11} and 10^{-10} g/f.p. (Kaissling and Priesner 1970).

The fraction of molecules on the filter paper that was released per s, given an airflow of 100 ml/s, was determined with loads of 10^{-8} to 10^{-4} g/f.p. The fraction was 1/60,000 at 10^{-8} and 10^{-7}g/f.p. This value was extrapolated for the load at the behavioural threshold. In our setup the concentration of stimulus molecules decreased on the way from the outlet of the airflow system to the antenna. The fraction of molecules released from the filter paper that was adsorbed on the antenna was 1/150, determined with loads of 10^{-6} g/f.p., or higher.

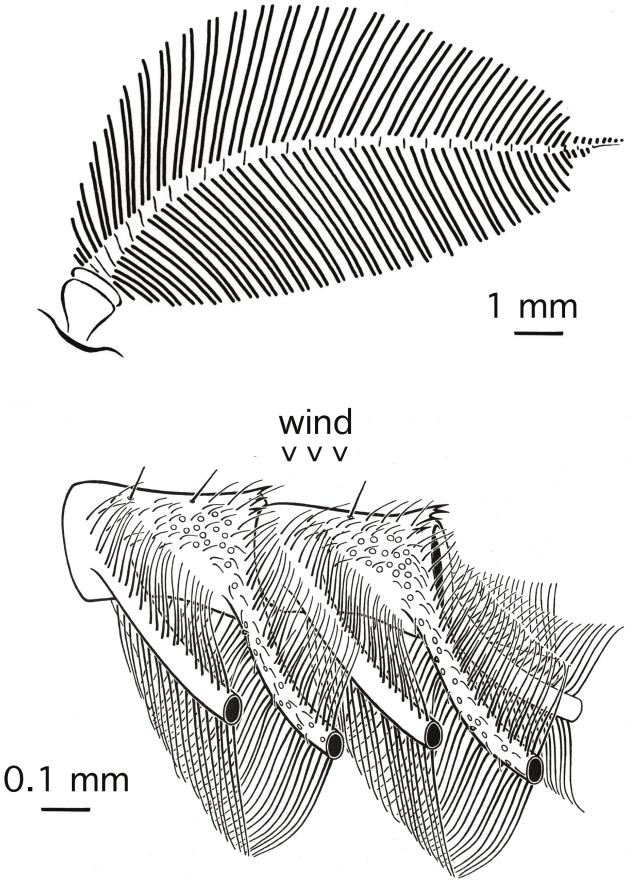

Fig. 3.1. Antenna of the male saturniid moth Antheraea polyphemus. Upper panel: Schematic view of the antenna. Each antennal stem segment has four side branches. Lower panel: Two antennal segments enlarged with different types of sensilla. The numerous, long olfactory hairs contain two or three receptor neurons responding to two or three components of the female pheromone.

With a load of 10^{-11} g/f.p. the concentration (c) of bombykol in air was 3,000 molecules/ml when the air stream velocity (v) was 57 cm/s as measured by means of a thermistor. Of the odour flow (*c x v x a*) (molecules/s) passing an area (a) equal to the outline area of the *Bombyx* antenna 27% was adsorbed on the antenna (Kaissling 1971). The air flow (air volume/s) through the actual antenna was most likely not more than 30% of the free air flow (through an area equal to the antennal outline area). A transmittance of about 30% of the free air flow may also be estimated for the much larger antenna of the male *Antheraea polyphemus* from measurements of air-stream velocity in front of and behind the antenna (Figures 3.1 and 3.2). The fraction

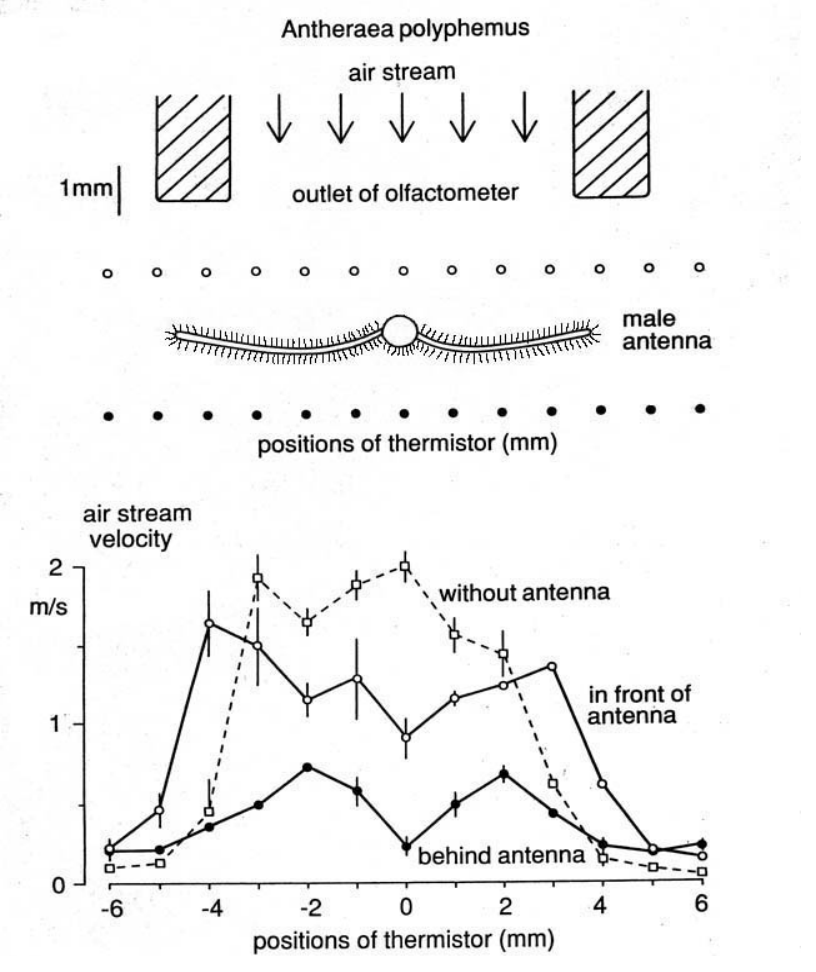

Fig. 3.2. Airflow at the antenna of the male moth Antheraea polyphemus. Air was blown from a glass tube towards the antenna. Scheme of the glass tube, antenna, and thermistor positions drawn to scale. By means of a thermistor (0.2 mm diameter) the airstream velocity was measured without the antenna (open sqares and dashed lines), in front of the antenna (open circles), and behind the antenna (dots).

of molecules passing an area equal to the antennal outline area that was adsorbed on these antennae was 32% (Kanaujia and Kaissling 1985). Thus we may conclude for both species of moths that from the air passing the antenna itself all pheromone molecules were caught.

In males of *Bombyx mori* (Steinbrecht and Kasang 1972) and *Antheraea polyphemus* (Kanaujia and Kaissling 1985) we determined the fraction of molecules caught by the antenna that was adsorbed on the long olfactory hairs (*sensilla trichodea*). The hollow, fluid-filled hairs are 2-3 micrometer thick and 100-300 micrometer long and house the sensitive dendrites of the pheromone receptor neurons. After 10-s exposure of single antennal branches to strong stimuli (10^{-4} g of ^{3}H-labeled pheromone/f.p.) the hairs were immediately (within 1 min) separated from the branch. 80% of the total radioactivity adsorbed was found on the hairs.

These findings show that the structure of the antenna, including the dimensions and arrangement of the olfactory hairs, is ideally tuned to the diffusion of odour molecules in air. It can be calculated that due to its thermal movements an odour molecule on its way through the antenna would hit the antennal hair surface about 100 times if it were reflected upon hitting. The design of antenna and hairs creates, as it were, an olfactory lens concentrating the stimulus and direct it to the sensory cells.

So far the exact chemical composition and structure of the hair surface and the pore tubules of the hair wall are unknown. Certainly the outer epicuticular layer is highly waterproof. If one damages the hair locally using a small laser beam one can - under microscopical control - see an air bubble growing starting from the point of hitting the hair.

3.3 Transport of Molecules on the Antenna

Following a strong concentration gradient, the pheromone molecules move along the hairs towards the body of the antennal branch. The velocity of this process can be measured if the hairs are cut at different times after exposure to ^{3}H-labeled pheromone. Within minutes the measured radioactivity decreased on the hairs while increasing on the antennal branch. From these measurements we determined a diffusion coefficient D of 50 $\mu m^2/s$ for the movement of pheromone on the hairs of *B. mori* (Steinbrecht and Kasang 1972). The diffusion coefficient was 90 $\mu m^2/s$ for air-filled hairs of dried antennae of *A. polyphemus*, and 30 $\mu m^2/s$ for intact hairs of fresh antennae (Kanaujia and Kaissling 1985). Modeling diffusion in *A. polyphemus* (Kaissling 1987; unpubl.) we use D = 90 $\mu m^2/s$ for the movement of the stimulus molecules along the hair surface and through the pore tubules, but D = 30 $\mu m^2/s$ for the diffusion through the sensillum lymph within the hair lumen towards the receptor neuron. The latter coefficient is expected for a protein molecule of the size of the pheromone binding protein (PBP) in water. Since the quantitative model of perireceptor and receptor events reveals that 83% of the pheromone adsorbed is bound to the PBP within less than 3 ms, we can conclude that the longitudinal movement of the ^{3}H-labeled pheromone represents the movement of the pheromone-PBP complex (Kaissling 2001; unpubl.). The remaining 17% of pheromone molecules are enzymatically degraded inside the hair lumen and may no longer function as stimulants.

With the above-mentioned diffusion coefficients the modeled delay of the molecule arrival at the receptor cell is about 10 ms after adsorption at the olfactory hairs (Kaissling 2001, Figure 3.7B). This fits to the minimum delay of the receptor potential, the first bioelectrical response of the receptor neuron, as measured after stimuli of high intensity. At weak stimulation the average delay of the responses is a few hundred ms due to the chemical reactions of the stimulus molecules including their interaction with the receptor molecules (Kaissling 2001, and unpubl.).

3.4 Cellular Transduction

At low stimulus intensities about 25% of the pheromone molecules adsorbed on the antenna elicit nerve impulses of the receptor neuron (Kaissling 1987). Modeling reveals that – besides the 17% enzymatically rapidly degraded molecules - more than half of the molecules adsorbed must be lost due to the - still hypothetical - odorant deactivation on the hairs (Kaissling 2001). The 25% fraction of effective molecules was determined by radiometric measurements and by counting the nerve impulses at low stimulus intensities such that about one nerve impulse is elicited per receptor neuron by a one-s stimulus (3x10-10 g of bombykol/f.p.). At and below this stimulus intensity one pheromone molecule is sufficient to elicit a nerve impulse (Kaissling and Priesner 1970).

The first responses of the receptor neuron to a single pheromone molecule are one or a group of small depolarizations (elementary receptor potentials, ERPs) (Kaissling and Thorson 1980; Kaissling 1994). The single ERPs with amplitudes of 0.1 – 1 mV in extracellular recordings last about 10 ms and may trigger firing of one, seldom more than one nerve impulse. Quantitative modeling suggested that *in vivo* it is the odorant-PBP complex rather than the free pheromone which interacts with the receptor molecule (Kaissling, 2001). The ternary complex PBP-pheromone-receptor may one or several times turn into an active state before it finally dissociates (Minor and Kaissling 2003). Each activation causes – via an intracellular cascade of signal processes – a transient conductance increase of about 30 pS as reflected in the ERP.

3.5 Processing in the Central Nervous System

The extreme sensitivity of the receptor neurons is combined with a most efficient processing of their responses by the central nervous system. Via the axons of the receptor neurons the nerve impulses are conducted to the antennal lobe, the first synaptic station of the central olfactory pathway in insects. The axons of the pheromone receptor neurons terminate on local interneurons and projection neurons (PN) of the macroglomerular complex (MGC) (Hildebrand 1996). The silk moth has 17,000 bombykol receptor neurons per antenna (Steinbrecht 1970) and 34 projection neurons connecting the MGC with higher centres of the central nervous system (Kanzaki et al. 2003). Since there are also 17,000 bombykal receptors the messages of at least 1000 (in the hawk moth *Manduca sexta* up to 10,000) receptor neurons finally converge to one projection neuron.

The convergence of receptor neurons lowers the threshold of pheromone detection by integrating the nerve impulses (spikes). Since the receptor neurons occasionally fire nerve impulses without stimulation, this background activity produces the noise which needs to be distinguished from the signal, i.e. the stimulus-induced activity. The spikes of single receptor neurons were counted every 0.1 s for two s after stimulus onset. The background frequency (f_{bg}) in spikes/s counted after control stimuli with clean air was subtracted from the frequency counted at pheromone stimulation in order to obtain the stimulus-induced frequency (f_{st}). Most of the behavioural responses (wing vibration) started within the first two s after stimulus onset, with an average delay of 0.2 s, the integration time (t_i) of the central nervous system. Since the background frequency of the bombykol receptor neuron (f_{bg} = 0.0855 spikes/s) has a random distribution (Kaissling 1971), its variability represents the noise that determines the recognizability of the signal. The noise is proportional to the square root (sqrt) of f_{bg}. Also the stimulus-induced frequency (f_{st}) was shown to be randomly (Poisson) distributed at stimulus intensities eliciting less than three nerve impulses per stimulus (Kaissling and Priesner 1970). For a number (n) of receptor cells the noise is

$$\sqrt{n \cdot t_i \cdot f_{bg}} \tag{3.1}$$

while the signal is

$$n \cdot t_i \cdot f_{st} \tag{3.2}$$

and the signal-to-noise ratio is

$$f_{st} \cdot \sqrt{\frac{n \cdot t_i}{f_{bg}}} \tag{3.3}$$

With a load of 10^{-11} g of bombykol /f.p. (10^{-10} g/f.p.) 40% (80%) of the males responded with wing vibration (at 21°C). At these loads we found f_{st} = 0.0145 (0.1545) spikes/s (from Tab. 2 in Kaissling and Priesner, 1970). For a convergence of 17,000 receptor neurons we find from Eq. 3.3 and with f_{bg} = 0.0855 spikes/s a signal-to-noise ratio of 3 (31). This shows that the processing in the CNS works near the theoretical limit.

The exact pattern of neuronal connections in the antennal lobe and the mechanism of signal/noise detection in the CNS are unknown. If we assume a minimum convergence, i.e. that each PN (directly or via interneurons) receives input from 1000 receptor neurons, the signal-to-noise ratio would be smaller than calculated above for an input from 17,000 neurons: For the 40% (80%) behavioural threshold we find a ratio of 0.7 (7.5). In this case the signal-to-noise ratio would be below the significant level of 3, at least for the 40% threshold. Consequently higher centres would need to contribute to the signal/noise detection, by converging the messages delivered from the PNs to higher-order neurons.

Finally it should be mentioned that the bombykol concentration in air at the 40% (80%) behavioural threshold was 3,000 (30,000) pheromone molecules/ml of air, at an airstream velocity of 57 cm/s. Interestingly these moths with 17,000 receptor neurons/antenna are almost as sensitive as a dog for (other) odorants (1000 molecules/ml).

Since a dog may have 10,000-fold higher numbers of receptor neurons than the moth, its thresholds could be 100-fold lower than the one of the moth. It could be even lower since the dog´s integration time is probably larger than the one of the moth. It seems clear that factors other than the number of receptor neurons are important for a high sensitivity, such as a high effectiveness of molecule capture and conveyance to the sensitive structures, or a low background activity of the receptor neurons. The low threshold in dogs suggests that as in the moth single molecules are able to produce nerve impulses.

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Dedication

This paper is dedicated to Prof. Dr. Dietrich Schneider, who started the analysis of pheromone detection in the silkmoth, on the occasion of his 88th birthday.

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