The insect antenna is not a molecular sieve

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Summary. The deposition rate of 2 tritiated odorants onto insect antennae of different sizes and shapes is linearly proportional to the product of odorant concentration times the antennal surface area. This result challenges an assumption in a commonly accepted hypothesis that sensillar shape affects odorant deposition and that a single odorant molecule can initiate an action potential in a receptor cell. Consequently the 1-molecule-1-spike hypothesis bears reinvestigation.

Key words. Insect antenna; odorant deposition; 1-molecule-1-spike hypothesis.

About 2 decades ago it was hypothesized that if the sensitive olfactory hairs covering an insect antenna had a particular size and spacing, the resultant feathery shape would make the antenna a highly efficient molecular sieve[1–5]. Presumably, male insects that followed sex pheromone plumes emitted by females long distances away would have a selective mating advantage if their antennae had a feathery shape. The hypothesis did not explain why relatively few species have feathery antennae, and later studies[6] showed that mate-locating ability was determined primarily by searching behavior patterns. However, the original hypothesis is still commonly accepted[7–9]. It is the basis of a predominantly untested prediction, cited frequently by physiologists, that a single odorant molecule can induce a spike in a receptor cell.

This report presents evidence that the rate of collection of odorant by an antenna is independent of shape and can be estimated by a simple equation from chemical engineering theory. The results imply that natural selection factors other than sieving efficiency are important in determining the size and spacing of olfactory hairs, e.g. developmental factors[10]. The results question an assumption in the 1-molecule-1-spike hypothesis, which thus bears reinvestigation.

Specimens of 5 different insect species, *Apis mellifera* L., *Bombyx mori* L., *Lymantria dispar* (L.), *Plodia interpunctella* (Hübner), and *Trichoplusia ni* (Hübner), with different types of antennae were exposed to 2 tritiated odorants, *(Z)*-7-dodecen-1-ol acetate (ZDDA, a sex pheromone of *T.ni)* and *(Z,E)*-9,12-tetradecadien-1-ol acetate (ZETA, a sex pheromone of *P.interpunctella*). The pheromones were refined by thin layer chromatography and silicic acid column chromatography just before use. The specific activities were 0.804 and 0.951 Ci/mmole, respectively, as determined by gas chromatography (GLC) and liquid scintillation counting (LSC) using external standards. The insects were exposed in a cylindrical Plexiglas® tunnel 14.6-cm ID × 270-cm long. The downwind end rested inside a fume hood and the hood door was adjusted to give windspeeds of 10, 45, 90, 250, or 500 cm/sec along the tunnel axis as measured by a Hastings® hot-wire anemometer. Parafilm® disks were spaced uniformly throughout the tunnel to monitor the odorant distribution and examine the effect of substrate shape. Air samplers, 5-mm ID × 10-cm-long brass tubes stuffed with 35-mg cotton filters at each end, were connected to a vacuum pulling tunnel air at 250 ml/min to determine the odorant concentration. The samplers were inserted downwind, 5 cm above a tray with 30 pinned insects positioned at the tunnel axis. Cotton threads soaked in solutions of Vaseline®, Stickum®, hexane-soluble lipids from insect cuticle, beeswax, and polyethylene glycol were suspended across prongs at the front of the tray to test the effect of substrate composition. The odorant dispenser was a glass tube, coated with a 0.5 ml aliquot of odorant in hexane, through which air was passed at 50 ml/min.

Each test was begun by setting the tunnel windspeed. For a 2- to 4-min exposure period a dispenser dosed with 100 µg of odorant was inserted upwind, emitting along the axis. Immediately thereafter the radioactivity of the deposited odorant was counted. From 6 to 10 antennae of a single species were combined into one sample to obtain measurable radioactivity. The antennal samples, air sampler filters, disks, cotton threads, and control standards were individually oxidized in a Packard Model 306® sample oxidizer and their radioactivity determined by LSC. The efficiency of the air samplers was calculated from the ratio of front-to-back filters.

The test results are consistent with current chemical engineering theory, which treats odorant transport as a 2-stage process[11–13]. The first stage involves transport of odorant to a boundary layer near the organ surface by convective diffusion. This stage is velocity-dependent because turbulence enhances the mixing of odorant with air. The second stage, deposition to the surface by diffusion across the boundary layer[11–14] is a complex process that is difficult to describe by theoretical mass-transfer equations, but frequently it can be estimated empirically from the equation:

$$R = CSK, \qquad (1)$$

where R (moles/sec) is the net rate of deposition, C (moles/cm^3) is the odorant concentration, S (cm^2) is the surface area, and K (cm/sec) is a constant called the deposition velocity[11–13,15]. It should be noted that, once the odorant molecules reach the surface, they must diffuse to sensillar pores and then to the receptor cell before they can be detected.

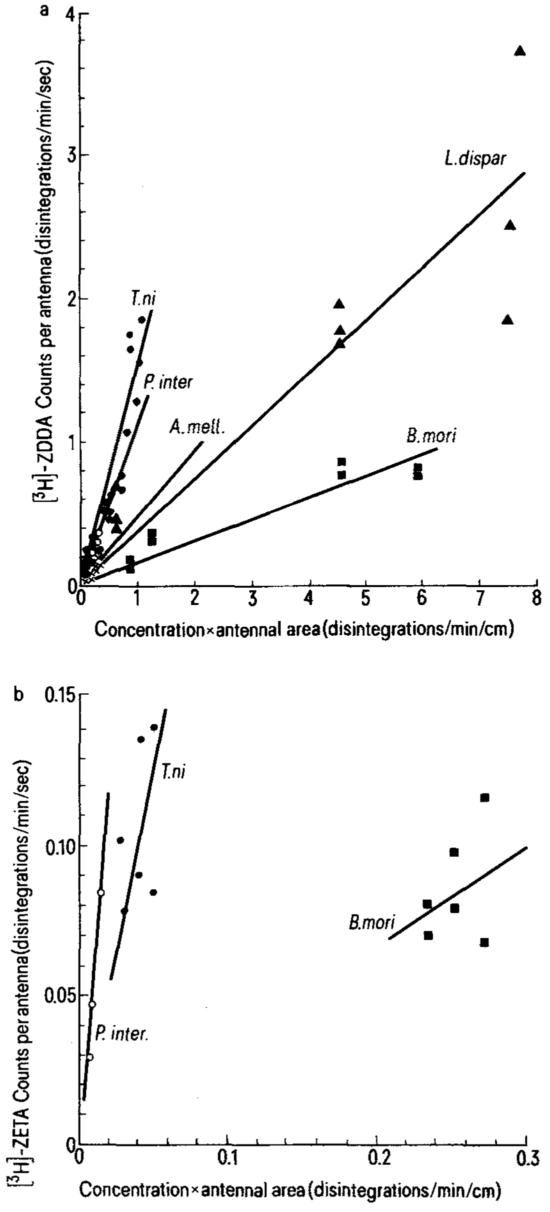
The major findings in the tests are shown in the figure where the radioactivity deposited per unit exposure period is plotted against the product of the concentration times the antennal area, the slope of the line being equal to K (table). Areas were calculated form published lengths and diameters (except for

Deposition velocities of ZDDA and ZETA: S (mm^2) is the surface area; K (cm/sec) ± SE is the deposition velocity determined by regression analysis of the means in the figure using Eq. 1

Substrate type	S (mm^2)	K (cm/sec) ZDDA	ZETA
Male insect antennae:			
A.mellifera	3.66	0.456 ± 0.032 (0.94)[a]	
B.mori	34.36	0.150 ± 0.013 (0.94)	0.304 ± 0.027 (0.96)
L.dispar	62.36	0.357 ± 0.035 (0.93)	
P.interpunctella	1.40	1.175 ± 0.055 (0.99)	5.098 ± 1.295 (0.89)
T.ni	5.36	1.551 ± 0.098 (0.90)	2.588 ± 0.261 (0.95)
Parafilm disk	126.67	0.399[b] ± 0.322	

[a] Coefficient of determination, r^2; [b] Calculated as the mean of 6 replications of 32 samples; the large SE is due to variation in odorant distribution at the upwind end of the tunnel.

P. interpunctella, which were means of 5 antennae) by idealizing the flagella (main shaft), the flagellar branches, if any, and the sensory hairs as cylinders[16]. The effect of velocity was not statistically significant so the data from all velocities were pooled. The values of K vary over an order of magnitude with a mean of about 1 cm/sec. The range is considerably smaller than the 4 or more orders of magnitude variation of odorant concentration typically encountered in odorant puffs in the field. Consequently, the odorant concentration, not the deposition velocity, is the most important factor affecting odorant detectability. Because K = 1 cm/sec is comparable to the reported values for many other chemicals[11-13], it can be used as an estimate of the deposition velocity for other odorants to simplify the calculation of behavioral thresholds and to predict levels of behavioral responsiveness[15]. Thus the differences in antennal shape and the size and spacing of olfactory hairs do not appear to affect the deposition velocity significantly.



a Mean rate of deposition of [^{3}H]-ZDDA onto 3–5 pair of antennae at different concentrations (the mass is in units of disintegrations/min). Several means used in the regression analysis are hidden by the close spacing of points: 6 of 30 means for *T. ni,* 8 of 15 for *A. mellifera,* 1 of 9 for *B. mori; b* Mean rate of deposition of [^{3}H]-ZETA onto 5 pair of antennae; Symbols: ●, *T. ni;* ○, *P. interpunctella;* x, *A. mellifera;* ▲, *L. dispar;* ■, *B. mori.*

The results of the disk and thread tests provide additional support for Eq. 1. Antennal and disk deposition velocities are not significantly different. Substrate composition is significant because deposition to the threads with a water-soluble coating was significantly reduced relative to deposition onto threads with the hexane soluble coatings. However, this effect is independent of antennal shape. The disks can be expected to have the same deposition velocity as the antennae because the chemical composition of the disks and the antennal surfaces are similar.

The error in the hypothesis predicting a shape effect is the assumption that all odorant molecules hitting a surface stick to it[15]. This error may not affect the 1-molecule-1-spike hypothesis[1-5]; nonetheless, it should be reformulated under more appropriate assumptions.

The impact of these findings is evident in consideration of the functional morphology of olfactory organs. Earlier hypotheses considered the feathery antenna to have evolved because it would be a more efficient sieve per unit area[1-5]. We argue that feathery insect antennae merely provide greater surface to support more odorant-sensitive hairs than do filiform antennae. When high sensitivity to a particular odorant was a significant factor in the reproductive fitness of an organism, the evolution of olfactory receptor dendrites tended to maximize surface to volume ratio. After physiological limitations on length and diameter of dendrites were approached, sensitivity to an odorant could be increased only by increasing the total number of receptor neurons. Apparently, other natural selection factors dictate evolution of a particular antennal type, including perhaps the number of sensilla that the antenna supports, an optimization of the ratio of the total surface area to the surface area of the sensilla, or factors affecting mechanical strength.

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