

Stimulus–Response Relationships of Insect Olfaction: Correlations Among Neurophysiological and Behavioral Measures of Response

R. W. MANKIN AND M. S. MAYER

*Insect Attractants, Behavior, and Basic Biology Research
Laboratory, Agricultural Research Service, United States
Department of Agriculture, Gainesville, Florida 32604, U.S.A.*

(Received 15 September 1981, and in final form 12 August 1982)

The inter-relationships among parameters of odorant concentration, the responses evoked in single olfactory receptor neurons, the whole-animal behavioral threshold, and the intensity of the behavioral response are examined for insect olfaction from the perspective of a new phenomenological model of the perceived intensity of single odorants. Electrophysiological and behavioral data compiled from diverse insect studies are analyzed in detail to determine the utility of the model for interpreting these studies and for designing new studies. The analysis of electrophysiological data indicates that the response of olfactory receptor neurons can be expressed as a power function of the odorant concentration. The behavioral thresholds to sex pheromone, predicted by the model from direct calculations using phenomenological parameters, are in good agreement with thresholds measured by bioassay. It is also shown that the interpretation of behavioral bioassay data in terms of concepts introduced by the model provides more information about the behavioral response than provided by the currently popular probit analysis. In addition, aspects of the physiological significance of power functions are discussed, and new methods are presented for distinguishing background activity from activity evoked in single olfactory neurons by an odor at threshold levels.

1. Introduction

The study of behavioral stimulus–response relationships in multi-cellular organisms is complicated by several factors. First, there is only limited knowledge about the transduction of stimuli into action potentials by peripheral sensory cells, and about the interactions among neurons in the central nervous system (CNS). Also, the ability of even the most rudimentary nervous system to process and integrate stimuli from several sensory modalities multiplies greatly the number of ways an organism can respond to stimuli from any single modality. This problem often confounds the

The first part of this study appeared in *J. theor. Biol.* (1983) **100**, 123.

design of behavioral experiments. Finally, there are a multitude of obscure interactions between an organism's genetic makeup and its environment. These interactions control the physiological processes underlying observable behavioral traits and must be understood thoroughly before the organism's selectivity in the stimuli that it perceives and the manner in which it responds can be interpreted properly.

Notwithstanding such complexities, it is practicable to model the process of olfaction on the basis of known relationships between external stimuli and single peripheral sensory neuron responses, between the summation of the peripheral sensory neuron responses and perception, and between the intensities of perception and the behavioral response. The preceding report introduced such a model (Mankin & Mayer, 1983). The model stimulus-response relationships characterize the olfactory neuron response as a power function of the odorant concentration. The perceived intensity of the odor in the CNS corresponds to the level of excitation at the sensing switch of the model. The level of excitation is directly proportional to the summed responses from the peripheral olfactory receptor neurons at a particular locus in the CNS, the central processor. Finally, the intensity of the behavioral response is characterized to be directly proportional to the level of excitation, i.e. the perceived intensity. This report addresses the question whether such a model has utility for explaining observed behavioral and neurophysiological processes despite its simplicity.

The relatively stereotypical responses of an insect to its sex pheromone, a chemical or group of chemicals eliciting a range of sexual activity, is an example where many of the complexities noted above are mitigated. The olfactory neurons on the insect antenna that respond to sex pheromone can often be distinguished from other olfactory neurons by the morphology of the olfactory hairs they innervate. They are easily counted and are accessible to the electrophysiologist (Mayer, Mankin & Carlyle, 1980). In some insects particularly sensitive to their sex pheromone, the majority of the olfactory hairs bear pheromone-sensitive neurons (Kaissling, 1971). Further, it has been demonstrated that the pheromone-sensitive neurons in some insects converge at a single locus called the macroglomerulus (Boeckh *et al.*, 1976; Boeckh & Boeckh, 1979; Hildebrand *et al.*, 1980). The macroglomerulus is analogous to the central processor of the model, which collects and sums the signals from all of the individual sensors. The remainder of the insect CNS and the motor neurons are analogous to the sensing switch. The ease of counting and access to the pheromone-sensitive neurons, the nature of their connections within the CNS, and the stereotypical behavioral response to the pheromone all suggest that the insect pheromonal response is a good test of the utility of the model.

The goodness-of-fit of the model to insect olfactory responses depends primarily on the accuracy of three hypothesized relationships. The first describes the response of single olfactory receptor neurons in terms of the odorant stimulus intensity, as discussed in sections 2 and 3. The second relationship, discussed in sections 4 and 5, calculates the behavioral threshold, the minimum concentration of odorant inducing a criterion behavioral response. The results from the electrophysiological and behavioral analyses in sections 2 and 4 are then applied to a consideration of how well the electrophysiological response at the behavioral threshold can be distinguished from the spontaneous background action potentials. The third hypothesized relationship, considered in section 7, estimates the intensity of a stereotyped behavioral response in terms of the odorant stimulus intensity.

Potentially, the model has considerable utility for the design, analysis, and comparison of insect bioassays. For example, it permits the pheromone stimulus levels to be compared in terms of physiologically identical units, such as molecules per receptor neuron per second, rather than less interpretable units like μg dose on filter paper. Also, it permits bioassays to be analyzed in terms of a graded response intensity rather than simply a quantal behavioral threshold. It will be shown that such an analysis provides more complete information about the behavior than the usual threshold analysis. The model also has some additional utility in enabling behavioral thresholds for sex pheromone to be calculated from parameter values already given in the literature for those cases where pheromone bioassays or electrophysiological measurements are difficult to perform.

2. The Stimulus-Response Relationship for a Single Pheromone Receptor Neuron

In deriving the model in the previous report, it was assumed that the rate of generation of action potentials by an olfactory neuron is a power function of the rate of adsorption of odorant to the exterior surface of the organ. Another assumption was that the rate of adsorption is linearly proportional to the odorant concentration in the stimulus air. Mathematically, these relationships are expressed by the equation (see equations (1) and (2), Mankin & Mayer, 1982):

$$M_p = b_1(K_c C + b_2)^b \quad (1)$$

where M_p (number/stimulus interval) is the mean rate of generation of action potentials by an olfactory neuron, b_1 , b_2 , and b are empirically

determined regression constants, C (moles/cm³) is the odorant concentration in the stimulus air, and K_c (cm³/mole interval) is a constant of proportionality between the odorant concentration and the rate of deposition of odorant molecules to the olfactory organ. In this section a least-squares analysis of electrophysiological recordings from single olfactory receptor neurons in male *Bombyx mori* (L.) (Kaissling & Priesner, 1970) will be used to test equation (1). The sex pheromone of this insect, (*Z,E*)-10,12-hexadecadien-1-ol, is hereafter called bombykol. The findings here will be applied to the calculation of the pheromonal response threshold in section 4.

The constant of proportionality in equation (1), K_c , is calculated by an equation from sections 2 and 6 of Mankin & Mayer (1983):

$$K_c = N_a t_i SK, \quad (2)$$

where N_a is Avogadro's number, 6.02×10^{23} /mole, t_i (sec/interval) is the duration of a stimulus interval, S (cm²) is the effective surface area from which the olfactory neuron collects odorant, K (cm/sec) is an empirically determined deposition velocity, and the units of K_c are cm³/mole interval. The stimulus interval is set by the experimental procedure, and the surface area is determined by the external morphology of the olfactory organs, called sensilla. We estimate the parameters in equation (2) to be $t_i = 2$ sec, $S = 6 \times 10^{-6}$ cm² and $K = 1$ cm/sec, according to which $K_c = 7.2 \times 10^{18}$ cm³/mole interval as discussed in the Appendix. The reader should bear in mind that, because of the limited precision of the estimates for S and K , calculations involving these parameters can only be estimated to within about a factor of five.

The values of M_p in equation (1) corresponding to given levels of bombykol cannot be determined directly from the data of Kaissling & Priesner (1970). They are calculated in Table 1 by a Poisson analysis of the time distribution of action potentials at each stimulus level (see section 6, Mankin & Mayer, 1983). The table lists values of N_x and M_p , where N_x is the number of stimulus intervals in which exactly x action potentials occur, x is an integral number of action potentials, 0, 1, 2, . . . , and M_p is the mean number of action potentials evoked from a single cell during a stimulus interval. The latter is calculated by the equation (Steel & Torrie, 1960):

$$M_p = \ln(N/N_0). \quad (3)$$

Equation (3), derived from the Poisson equation, is strictly valid only for a Poisson distribution. Although the frequency of occurrence of action potentials from a neuron does not strictly follow a Poisson distribution in

TABLE 1

The neurophysiological response of bombykol-sensitive olfactory neurons as a function of bombykol concentration in the stimulus air. Data are retabulated from Table 2 of Kaissling & Priesner (1970). The stimulus interval was 2 sec and 165 different cells were tested. Nomenclature: N_aC (molecules/cm³) is the odorant concentration in the stimulus air calculated from the calibration of a dose of 3×10^{-6} µg on filter paper corresponding to a concentration of 1000 molecules/cm³. N is the number of stimulus intervals, N_x is the number of stimulus intervals in which exactly x action potentials were recorded, and M_p (number/interval) is the mean rate of generation of action potentials from a single neuron, calculated from equation (3) in the text

N_aC	N		N_x				M_p $x=4$
			$x=0$	$x=1$	$x=2$	$x=3$	
Control	1070	916	132	17	3	2	0.16
310	610	521	77	7	5	0	0.16
3100	866	716	129	18	3	0	0.19
31 000	895	533	305	47	9	1	0.52
310 000	807	26	96	124	142	125	3.44

a rigorously statistical sense (see section 6 and Cox & Lewis, 1966), the deviations of the Kaissling and Priesner data from the Poisson distribution do not seriously affect the validity of the model, as will be shown later. We confirmed the applicability of equation (3) to the *B mori* data by calculating several values of M_p directly, dividing the total number of action potentials by the total number of intervals. The mean spontaneous activity calculated directly was 0.17 action potentials/interval, which was in good agreement with the calculation of 0.15 from equation (3). The difference, which is not statistically significant, is due to the fact that the occurrences of action potentials tended to correlate positively with the occurrence of previous action potentials.

The regression constants for equation (1) were calculated from the data in Table 1 by a modified Newton-Gauss least-squares procedure (Goodnight, 1979). In addition, regression constants were calculated for hyperbolic and logarithmic functions that have been considered in other analyses (see section 6, Mankin & Mayer, 1983). The constants for all three regressions are listed in Table 2 and the regressions are plotted in Fig. 1.

The power function appears to best fit the data, albeit all three regressions have high coefficients of determination, r^2 . The high values of r^2 are due in part to the low number of data points relative to the number of regression

TABLE 2

Equations for the regression of the rate of action potentials on odorant concentration from the data of Table 1. Nomenclature: M_p (number/interval) is the mean rate of generation of action potentials by a bombykol-sensitive olfactory neuron; b , b_1 - b_6 are regression constants (\pm standard error); C (moles/cm³) is the bombykol concentration; $K_c = N_{at}SK = 7.2 \times 10^{18}$ cm³/mole interval; and r^2 is the coefficient of determination

Power function	Hyperbolic function	Logarithmic function
$M_p = b_1(K_c C + b_2)^b$	$M_p = \frac{b_3 K_c C}{b_4 + K_c C} + 0.1554$	$M_p = b_5 \ln(K_c C + b_6)$
$b_1 = 0.9709 \pm 0.0036$	$b_3 = -344\ 089.9 \pm 2079.6$	$b_5 = 2.1788 \pm 0.1114$
$b_2 = 0.1388 \pm 0.0020$	$b_4 = -389\ 889.7 \pm \dagger$	$b_6 = 1.0263 \pm 0.1152$
$b = 0.9357 \pm 0.0025$	$r^2 = 0.999\ 902$	$r^2 = 995\ 158$
$r^2 = 0.999\ 999$		

† Calculation of this standard error was beyond the capability of the computer program.

Note: The standard errors here are indicative of the precision of the regression line only, and are not indicative of the accuracy of the model as a whole because the values of S and K are uncertain to within about a factor of five.

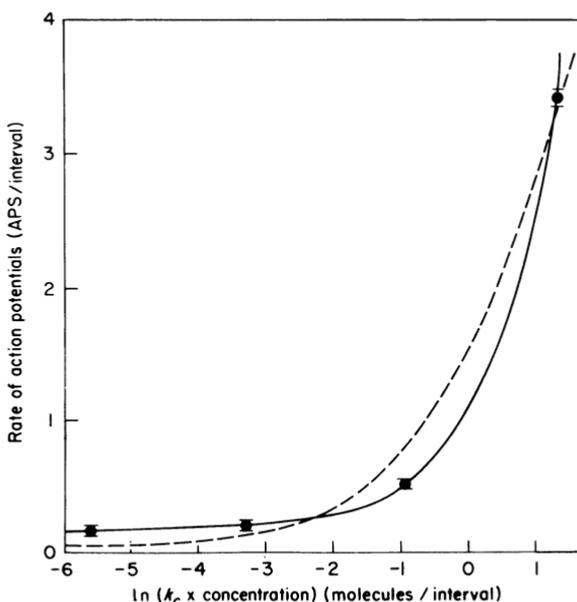


FIG. 1. The rate of generation of action potentials by a bombykol-sensitive receptor neuron as a function of the bombykol concentration. The smooth line designates the hyperbolic and power functions of best fit to the data, indicated by closed circles. The standard errors of the data points are indicated by bars. The dashed line indicates the logarithmic function of best fit. $K_c = 7.2 \times 10^{18}$ cm³/mole interval.

constants. We digress briefly in the next section to consider how the magnitudes of the regression constants for *B. mori* olfactory neurons differ from those of other olfactory power functions and to discuss the physiological implications of the differences. Later sections deal with applications of the *B. mori* pheromonal power function in determining the behavioral threshold and the electrophysiological response at the threshold.

3. Effect of Stimulus Range on Regression Constants

In most of the sensory modalities there is a systematic relationship between neural power functions that measure evoked potentials, such as the one in Table 2, and power functions that measure perceived intensity (Stevens, 1975). Usually the exponent, b , in a neural power function is smaller than the exponent in the corresponding perceived intensity power function. Because the exponent in the power function for human olfaction is often 0.7 or less (Cain & Engen, 1979), it is somewhat surprising that the exponent of the neural power function in *B. mori* is as large as 0.9 (Table 2). In addition, b_2 is usually negative in psychophysical functions, whereas it has a value of +0.14 in the case of *B. mori*. The differences between the neural and psychophysical functions could be due to a number of factors, but a particularly significant one may be the difference in the range of stimulus intensities examined.

We investigated the effect of the range of concentration on the neural power function in *B. mori* by referring to Fig. 3 in Kaissling & Priesner (1970). This figure presents examples of recordings from a single bombykol-sensitive olfactory receptor neurons exposed to concentrations from zero up to 3.1×10^8 molecules/cm³. Three data points in addition to those in Table 1 can be calculated from the examples: $M_p = 12$ action potentials/interval at $N_a C = 3.1 \times 10^6$ molecules/cm³, $M_p = 37$ at $N_a C = 3.1 \times 10^7$, and $M_p = 55$ at $N_a C = 3.1 \times 10^8$. If these are combined with the original data in the regression analysis, the regression constants of best fit become $b_1 = 1.7 \pm 2.4$, $b_2 = -0.0025 \pm 0.50$, and $b = 0.47 \pm 0.18$, with $r^2 = 0.835$. Note that the new b is lower than the value of 0.7 commonly found for human olfaction. The new neural power function thus appears to be in better agreement with the human perception power function than the original power function in Table 2. This agreement is misleading, however. Comparing the two curves for the original and combined data in Fig. 2, we see that the original curve fails to fit the last three data points, while the plunge of the curve for the combined data at $\ln(K_c C) = -2.7$ is obviously an artifact of the regression analysis.

The fact that the exponent, b , decreases when the additional points at higher stimulus intensities are added to the data base suggests that the

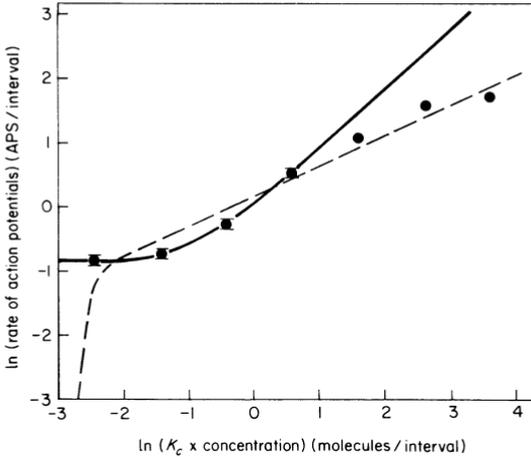


FIG. 2. Comparison of the power function calculated in Table 2 (solid line) with the power function calculated from data that includes the responses of olfactory neurons to high concentrations of bombykol (dashed line). The measured responses are indicated by solid circles. The standard errors of the measurements at each dose, indicated by the bars, are known for only the first four points because the last three points are single examples.

original function in Table 2 is valid at low stimulus intensities and that b begins to decrease after the odorant concentration rises above some critical level (e.g. Wasserman, Felsten & Easland, 1979). If such were the case, the difference in the exponents of the neural and psychophysical power functions might disappear if the stimuli were restricted to values within two or three orders of magnitude above the threshold. The commonly obtained negative value of b_2 may be an artifact caused by a decrease in b at higher stimulus intensities. We will assume for the remainder of the report that the power function listed in Table 2 is the most valid of the two functions, at least in the range of stimulus intensities from zero to 3.1×10^5 molecules/sec.

It remains to consider the goodness-of-fit of the model to the two other hypothesized relationships specified in the introduction. The next three sections deal with calculation of the behavioral threshold to sex pheromone, after which will follow a discussion of how insect pheromone threshold bioassays can be interpreted in terms of power functions.

4. The Behavioral Threshold to Sex Pheromone

The behavioral threshold, i.e. the minimum concentration of odorant necessary to elicit a criterion behavioral response, is currently the principal

measure of olfactory acuity in insects. The threshold for the sex pheromone is a particularly critical parameter in models of the attraction of insects to sex pheromone-baited traps (Mankin *et al.*, 1980a). It would be of practical benefit for the development of sex pheromones as agents of insect control if the threshold could be estimated without resort to expensive, time-consuming bioassays. Consequently, a primary goal during construction of the olfactory perception model was to derive an equation calculating a threshold directly from phenomenological parameters whose magnitudes were already known.

In deriving the threshold equation it was necessary to adopt three assumptions: (1) the total mean rate of transfer of spontaneous action potentials from the n olfactory receptor neurons to the central processor is $\mu = nM_s$; (2) the instantaneous rate of transfer varies about the mean with a standard deviation of $\sigma = \sqrt{nM_s}$; and (3) the probability of a spontaneous behavioral response is equal to p_0 , the probability that the total rate of transfer of spontaneous action potentials to the central processor rises above the level, $\mu + Z_0\sigma$, where Z_0 (number of action potentials/interval)^{1/2} is a constant equivalent to the unit normal deviate for p_0 . Experimentally, the magnitude of Z_0 is determined by measuring p_0 in a bioassay and then reading the Z value corresponding to p_0 from a table of the normal distribution.

An example of the calculation of Z_0 is obtained from bioassays of *B. mori*. Male *B. mori* respond to their sex pheromone initially by lifting their antennae and fluttering their wings (Schwinck, 1954). This response can be used as a criterion that an insect has detected the pheromone. Kaissling & Priesner (1970) found that a control stimulus elicited the criterion response with a probability of 0.04. If we assume that this probability is equal to p_0 , then the corresponding Z_0 in the normal distribution table is 1.75 (action potentials/interval)^{1/2}. It should be noted that the variation in Z_0 is relatively small compared to the variation in n , the number of receptor neurons, or C , the pheromone concentration, as can be seen in Fig. 2 of Mankin & Mayer (1983). If p_0 decreases to 0.001, Z_0 increases only to 3.09. A good general estimate might be $Z_0 = 2.0$ (action potentials/interval)^{1/2}.

The threshold equation derived in Mankin & Mayer (1983), subject to the three conditions above, is:

$$T = Z_0(M_s/n)^{1/2}/qK_c \quad (4)$$

where T (moles/cm³) is the behavioral threshold and the other symbols are as previously defined. In calculating T for *B. mori*, we chose to set $Z_0 = 1.75$ (action potentials/interval)^{1/2}, $M_s = 0.15$ action potentials/inter-

val, $q = 1.03$ action potentials/molecule, $K_c = 7.2 \times 10^{18}$ cm³/mol interval, and $n = 25\,600$ (see Kaissling, 1971). The result is $T = 5.79 \times 10^{-22}$ moles/cm³ = 349 molecules/cm³. This estimate is about half the measured threshold of 650 molecules/cm³ (Kaissling & Priesner, 1970). Such close agreement of observed and predicted thresholds is surprising, considering the simplicity of the model and the uncertainty in K and the other parameter values.

5. Calculation of Neurophysiological Parameters from Behavior and Morphology

In this section the reverse of the problem solved above is considered, how to estimate unknown neurophysiological parameters from known behavioral and morphological parameters. Two insects for which there is a sufficient data base to permit such calculations are the cabbage looper, *Trichoplusia ni* (Hübner), and the Indian meal moth, *Plodia interpunctella*

TABLE 3

Calculation of the spontaneous activity of single pheromone receptor neurons of three different insects from phenomenological parameters in equation 5. In each case the following parameter values are assumed: $q = 1$ action potential/molecule $t_i = 1$ sec, $K = 1$ cm/sec, $Z_0 = 1.75$ (action potentials/interval)^{1/2}

	Insect		
	<i>T. ni</i>	<i>P. interpunctella</i>	<i>B. mori</i>
Number of sensilla (n)	10 800 ^a	5000 ^b	25 6000 ^c
Sensillar surface area (S) (10 ⁻⁶ cm ²)	2.6 ^a	2.0 ^d	6.0 ^c
K_c (10 ¹⁸ cm ³ /mole sec)	1.6	1.2	3.6
Threshold (τ) (molecules/cm ³)	8000 ^e	16 500 ^f	650 ^g
Calculated M_s (action potentials/sec)	1.5	1.8	0.13
Measured M_s (action potentials/sec)	6.0 ^h	—	0.07 ^g

^a From Mayer *et al.* (1981).

^b Estimate, based on the size of the antenna relative to *T. ni*.

^c From Kaissling (1971).

^d Estimated by comparison with *T. ni* sensilla.

^e Sower *et al.* (1971).

^f Mankin *et al.* (1980b).

^g Kaissling & Priesner (1970).

^h Mean of 10 cells, S.E. = 1.2.

(Hübner), whose pheromones are (*Z*)-7-dodecen-1-ol acetate and (*Z,E*)-9,12-tetradecadien-1-ol acetate (*ZETA*), respectively. An estimate of the spontaneous activity of the receptor neurons is obtained by transposing equation (4) into

$$M_s = n(qK_c\tau/Z_0)^2, \quad (5)$$

where Y is the measured behavioral threshold, used as an estimate for T .

The calculated values for the spontaneous activities of pheromone-sensitive cells of three different insects are shown in Table 3 along with measured values. The calculated and measured values are in general agreement.

6. The Electrophysiological Response at the Behavioral Threshold

Usually the behavioral threshold is assumed to be two or three orders of magnitude lower than the electrophysiological threshold, the lowest concentration at which the electrophysiological response can be distinguished statistically from the background noise (Kaissling, 1971; Boeckh & Boeckh, 1979). In the model, the two thresholds are identical and, indeed, if the distribution of pheromone-induced action potentials in *B. mori* olfactory neurons at stimulus concentrations near the behavioral threshold is examined via Poisson analysis, some differences from the distribution of spontaneous action potentials are observed.

The pheromone-induced distribution is calculated from the total observed distribution of spontaneous and pheromone-induced action potentials by an equation based on the distributive property of the Poisson distribution:

$$P_{m_1+m_2}(x) = [(m_1 + m_2)^x x! / m_1^x m_2^x] P_{m_1}(x) P_{m_2}(x), \quad (6)$$

where m_1 and m_2 are any two means. This equation derives from the Poisson equation:

$$P_m(x) = m^x e^{-m} / x! \quad (7)$$

where $P_m(x)$ is the probability of occurrence of exactly x action potentials/interval when the mean is m . If m is set equal to $m_1 + m_2$, the result is equation (6).

In applying equation (6) for determining the distribution of odorant-induced action potentials, we first note that

$$M_0 = M_p - M_s, \quad (8)$$

where M_0 (number/stimulus interval) is the mean rate of generation of odorant-induced action potentials by an olfactory receptor neuron. Because

the distribution of action potentials closely approximates a Poisson distribution it is convenient to assume that

$$N_x/N = P_{M_p}(x), \quad (9)$$

and

$$n_{o,x}/N = P_{M_o}(x), \quad (10)$$

where N_x is the number of intervals in which exactly x action potentials occur, N is the total number of intervals, and $n_{o,x}$ is the number of intervals in which exactly x odorant-induced action potentials occur. Then, by setting $M_o = m_1$ and $M_s = m_2$ in equation (6) and combining it with equations

TABLE 4

Poisson distributions of the odorant-induced action potentials from bombykol-sensitive olfactory receptor neurons in B. mori at different bombykol concentrations. Nomenclature: N_aC (molecules/cm³) is the concentration of bombykol in the stimulus air; M_o (number/interval) is the mean rate of generation of odorant-induced action potentials calculated from Table 1 and equation (8); x is the number of action potentials per interval; $N_{exp,x}$ is the expected number of intervals in which exactly x odorant-induced action potentials occurred, calculated from Table 1, equations (8) and (10); $N_{obs,x}$ is the observed number of intervals in which exactly x action potentials occurred, calculated from Table 1, equations (3) and (11); χ^2 is the cumulative chi-square, calculated from the equation: $\chi^2 = \sum_{i=0}^x (N_{exp,i} - N_{obs,i})^2 / N_{exp,i}$

N_aC	M_o	x	$N_{exp,x}$	$N_{obs,x}$	χ^2
310	0.0023	0	608	608	0
		1	2	2	0
3100	0.0348	0	836	836	0
		1	29	28	0.03
		0	623	623	0
31 000	0.3629	1	226	250	2.55
		2	41	27	7.33
		0	30	30	0
		1	100	107	0.49
310 000	3.2796	2	164	136	5.27
		3	179	144	12.11
		4	146	121	16.39

Note: the precision of the calculated values is much greater than the precision of the model as a whole because other parameters in the model are known only to within about a factor of five.

(8)–(10), we obtain

$$n_{o,x} = N_x M_o^x M_s^x / [P_{M_s}(x)(M_o + M_s)^x x!]. \quad (11)$$

The values of N_x for $x = 0-4$ at each concentration of bombykol are listed in Table 1. The values of $n_{o,x}$ calculated by equation (11) from the N_x are shown under the $N_{\text{obs},x}$ heading in Table 4. For comparison the $n_{o,x}$ calculated by equation (10) are shown under the $N_{\text{exp},x}$ heading in Table 4.

The hypothesis that $N_{\text{obs},x}$ and $N_{\text{exp},x}$ are identical is tested by the cumulative χ^2 in the last column of the table (Steel & Torrie, 1960). At the 95% confidence level the critical values of χ^2 are 3.84, 5.99, 7.81, and 9.49 for 1, 2, 3, and 4 degrees of freedom, respectively. In this case the number of degrees of freedom is equal to x because there are $x + 1$ classes and because the mean of the Poisson distribution is calculated from the data. Thus, by the χ^2 criterion the observed and expected numbers

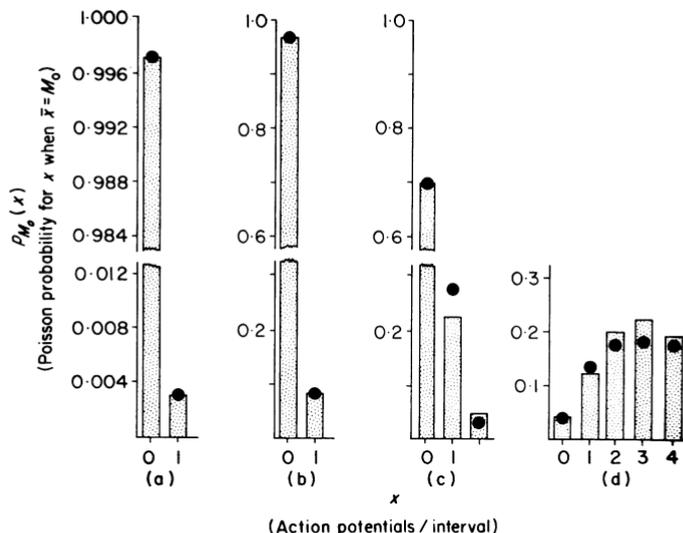


FIG. 3. Comparison of expected and observed distributions of action potentials (APS) per interval for the *B. mori* data in Table 4. Expected distribution is calculated by equation (10) and the observed distribution by equation (11). Nomenclature: $P_{M_o}(x)$ is the Poisson probability of obtaining exactly x APS per interval when the mean number of APS per interval is M_o . (a) $C = 310$ molecules/cm³; $M_o = 0.0023$ action potentials/interval. (b) $C = 3100$; $M_o = 0.035$. (c) $C = 31\ 000$; $M_o = 0.36$. (d) $C = 310\ 000$; $M_o = 3.28$.

tested within the class, $x = 1$, appear to be in agreement at each concentration of bombykol. For tests including the classes, $x = 3$ or 4, there is a poor statistical fit at the highest concentration. Also, at $N_a C = 31\ 000$ molecules/cm³ there is a poor fit for $x = 2$. The expected and observed

distributions are plotted in Fig. 3. The three discrepancies all lie in the direction that would be expected if facilitation of action potentials by preceding action potentials were to occur (Kaissling & Priesner, 1970).

Because the Poisson analysis yielded a nonzero value for M_o at even the lowest concentration of bombykol tested, 310 molecules/cm³, it could be argued that the electrophysiological threshold and the behavioral threshold are identical. This argument does not hold, however, when a least-significant-difference test is applied in comparisons between the control and the lowest stimulus levels. Used for these comparisons, such a test is not strictly valid from a statistical viewpoint because it assumes a normal rather than a Poisson distribution. Nonetheless, for lack of a better alternative, we can apply the test to obtain an approximate estimate of the minimum stimulus distinguishable from the noise. The lowest dose for which the mean response is significantly different from the noise at the $t_{0.05}$ level is the dose of 3100 molecules/cm³, less than an order of magnitude above the behavioral threshold, 650 molecules/cm³. It thus appears that the differences commonly reported between electrophysiological and behavioral thresholds are not inherent but simply reflect the effects of sample size and the method of analysis on the evaluation of mean responses to the stimulus and the noise.

7. Inferences about Insect Behavioral Bioassays

In section 6 of Mankin & Mayer (1982), it was hypothesized that the dose-response relationships for bioassays involving stereotyped behavior are power functions, such as

$$R = R_o(C - \tau)^\beta, \quad (12)$$

where R is some appropriate measure of response intensity, τ , R_o and β are regression constants, and C is the odorant concentration. It was further suggested that the exponent, β , is likely to be equal to or slightly less than that in the psychometric power function for perceived intensity of stimulus. If this is true, the phomonal dose-response bioassays that are commonly performed to determine phomone thresholds yield additional information about the intensity of phomone perception. Such information is of interest in its own right, but it has practical value as well. For example, models of the attraction of insects to sex phomone traps usually consider attraction in terms of a quantal response (Mankin *et al.*, 1980a). If the phomone concentration near the trap rises above a threshold level then, hypothetically, the insect always finds the trap. A more realistic model would grade the intensity of the attraction response with respect to the phomone concentration, perhaps by means of a power function.

The exponent, β , for such an "attraction" power function can be estimated by reference to bioassays like those done with *B. mori* by Kaissling & Priesner (1970), and *P. interpunctella* by Mankin *et al.* (1980*b*). We estimated the constants in the power function for *B. mori* at 17 and 21°C from the Kaissling & Priesner (1970) data by a nonlinear least-squares analysis. In these tests the pheromone stimulus was quantified in terms of μg dose on filter paper and the response in terms of percentage insects fluttering wings. The results are listed in Table 5. Similar calculations were done also for the bioassays of *P. interpunctella* in Mankin *et al.* (1980*b*). Here, the stimulus was quantified in terms of μg dose in the dispenser and the response in terms of percentage insects flying 3 m upwind.

TABLE 5

Regression constants in the power function of equation (12) for behavioral responses to pheromone by B. mori and P. interpunctella at different temperatures

Insect	Temperature (°C)	Regression constants \pm S.E.			
		R_0 (% response)	τ (μg dose)	β	r^2
<i>B. mori</i>	17 ^a	730 ± 290	-9.9×10^{-7} $\pm 9 \times 10^{-8}$	0.29 ± 0.05	0.98
	21 ^b	700 ± 460	-9.9×10^{-7} $\pm 2 \times 10^{-8}$	0.33 ± 0.03	
<i>P. interpunctella</i>	23 ^c	25.0 ± 6.4	-1.0×10^{-4} $\pm 0.5 \times 10^{-4}$	0.22 ± 0.09	0.99
	34 ^d	70.0 ± 6.2	-3.1×10^{-4} $\pm 2 \times 10^{-5}$	0.14 ± 0.03	

^a The dose-response function was calculated for the range of 10^{-6} to 10^{-3} μg bombykol on filter paper.

^b The function was calculated for the range of 10^{-6} to 10^{-4} μg bombykol on filter paper.

^c The function was calculated over the range of 10^{-3} to $10^{1.5}$ μg ZETA coated onto the inside of a glass dispenser.

^d The function was calculated over the range of 10^{-3} to 1 μg ZETA coated onto the inside of a glass dispenser.

These results are interesting in that, even though the response measures and the stimulus presentation methodologies differ in the two studies, three of the four values for β do not differ significantly from each other at the 95% confidence level. The differences that do occur lie in the direction that might be expected, i.e. the propensity to fly upwind is lower than the propensity to flutter wings. A good estimate for the exponent in an attraction behavior power function thus might be $\beta = 0.2$, with the exponent for the power function of perceived intensity being slightly higher. It should be

noted also that τ is approximately equal to the behavioral threshold calculated by the usual probit analysis procedures. Consequently, equation (12) may be useful for the analysis of other pheromonal bioassay data and trap catch data (see e.g., Fig. 1 in Beroza *et al.*, 1971), particularly because the exponent, β (which is an intrinsic response gain or compliance factor, see section 3, Mankin & Mayer, 1982), is independent of the stimulus and response units. Thus, the power function analysis points out underlying relationships among the thresholds and exponents (response compliance factors) in electrophysiological and behavioral measurements of olfactory perception.

8. Possible Improvements to the Model

The simplicity of the model is both an advantage and a liability. It is advantageous that the model can point out in simple terms some underlying principles relating electrophysiological and behavioral measurements of olfactory perception. It is also advantageous that several important psychophysical parameters can be estimated easily from the model relationships. However, to thoroughly understand discrimination, adaptation, habituation and complicated behaviors associated with attraction to a pheromone source (Mankin *et al.*, 1980a), it will be necessary to model more completely the activities of the individual olfactory neurons and the CNS. In addition, the process whereby odorant molecules diffuse from the surface of an olfactory organ to the underlying sensory dendrites could be modeled in greater detail to allow calculation of the electrophysiological response latency at the beginning of a stimulus presentation and the response overshoot at the end of a presentation. Getchell *et al.* (1980) have considered this with respect to vertebrate olfaction.

One improvement that can be made in the model now, based on the analysis of the data of *B. mori*, is a modification of the assumption that the response of an olfactory neuron is a power function of the stimulus intensity. This assumption either must be restricted to stimulus levels within 2–3 orders of magnitude of the threshold, or we must assume that the exponent, b , is not constant. It may be possible to accommodate this problem in the model by replacing b with a parameter that is a decreasing function of the odorant concentration.

Lastly, the model could be improved by considering the responses to stimuli that vary with time. Aspects of this problem have already been considered by Knight (1972).

A critical problem in verifying the model is the lack of reliable electrophysiological and behavioral measurements against which the model

calculations can be compared. Significant improvements in the model and the model concepts will come only after a larger data base has been obtained.

REFERENCES

- BEROZA, M., BIERL, B. A., KNIPLING, E. F. & TARDIE, J. G. R. (1971). *J. econ. Ent.* **64**, 1527.
- BOECKH, J., ERNST, K., SASS, H. & WALDOW, U. (1976). *Verh. dt. zool. Ges.* 1976, 123.
- BOECKH, J. & BOECKH, V. (1979). *J. comp. Physiol.* **132**, 235.
- CAIN, W. S. & ENGEN, T. (1969). In: *Olfaction and Taste III* (Pfaffmann, C., ed.). New York: Rockefeller University Press.
- COX, D. R. & LEWIS, P. A. W. (1966). *The Statistical Analysis of Series of Events*. London: Chapman and Hall.
- DEN OTTER, C. J. (1977). *J. comp. Physiol.* **121**, 205.
- GETCHELL, T. V., HECK, G. L., DESIMONE, J. A. & PRICE, S. (1980). *Biophys. J.* **29**, 397.
- GOODNIGHT, J. H. (1979). In: *SAS User's Guide, 1979 Edition* (Helwig, J. T. and Council, K. A., eds). Raleigh: SAS Institute, Inc.
- HILDEBRAND, J. G., MATSUMOTO, S. G., CAMAZINE, S. M., TOLBERT, L. P., BLANK, S., FERGUSON, H. & ECKER, V. (1980). In: *Insect Neurobiology and Pesticide Action (Neurotox 79)*. (Rickett, F. E., ed.). London: Society for Chemical Industry.
- KAISLING, K.-E. & PRIESNER, E. (1970). *Naturwiss* **57**, 23.
- KAISLING, K.-E. (1971). In: *Handbook of Sensory Physiology IV: Chemical Senses-Olfaction* (Beidler, L. M., ed.). Berlin: Springer-Verlag.
- KNIGHT, B. W. (1972). *J. gen. Physiol.* **59**, 734.
- MCMAHON, T. A. & DENISON, P. J. (1979). *Atmosph. Environ.* **13**, 571.
- MANKIN, R. W., VICK, K. W., MAYER, M. S., COFFELT, J. A. & CALLAHAN, P. S. (1980a). *J. chem. Ecol.* **6**, 929.
- MANKIN, R. W., VICK, K. W., MAYER, M. S. & COFFELT, J. A. (1980b). *J. chem. Ecol.* **6**, 919.
- MANKIN, R. W. & MAYER, M. S. (1983). *J. theor. Biol.* **100**, 123.
- MAYER, M. S., MANKIN, R. W. & CARLYSLE, T. C. (1980). *Int. J. Morph. Embryol.* **10**, 185.
- O'CONNELL, R. J. (1975). *J. gen. Physiol.* **65**, 179.
- SCHWINCK, I. (1964). *Z. vergl. Physiol.* **37**, 19.
- SOWER, L. L., GASTON, L. K. & SHOREY, H. H. (1971). *Ann. ent. Soc. Am.* **64**, 1448.
- STEEL, R. G. D. & TORRIE, J. H. (1960). *Principles and Procedures of Statistics*. New York: McGraw-Hill.
- STEVENS, S. S. (1975). *Psychophysics*. New York: John Wiley & Sons.
- VARESCHI, E. (1971). *Z. vergl. Physiol.* **75**, 143.
- WASSERMAN, G. S., FELSTEN, G. & EASLAND, G. S. (1979). *Science* **204**, 85.

APPENDIX

Calculation of K_c

In choosing the estimates for t_i , S , and K , in section 2 we evaluated the following evidence. The odorant stimulus in the study of Kaissling & Priesner (1970) was presented by placing a dose of bombykol onto filter paper and then passing an airstream over the paper onto male *B. mori* antennae for one second intervals. The neurophysiological responses from

single, bombykol-sensitive olfactory neurons were recorded for 2 sec rather than 1 sec, however, because there is a lag between the beginning of the stimulus and the arrival of odorant at the dendritic membrane of the olfactory neuron, as well as a lag between the end of the stimulus and the removal of odorant from the vicinity of the neuron. Consequently, a precise estimate for t_i cannot be determined. We set t_i to 2 sec with the caveat that this may overestimate slightly the actual stimulus interval.

The estimate for S , the effective surface area from which the olfactory receptor neuron collects bombykol, is set equal to the surface area of the sensillum overlying the receptor neuron because this is the surface adsorbing the molecules that eventually diffuse to the neuron. Two factors, nevertheless, make the actual value of S less than the sensillar surface area, $6 \times 10^{-6} \text{ cm}^2$ (Kaisling & Priesner, 1970). First, a small fraction of the adsorbed molecules diffuse off the sensillum to adjacent surfaces. Second, the sensillum is innervated by two or more receptor neurons, only one of which is sensitive to bombykol. Those molecules that diffuse to one neuron may be prevented from later contact with the other neuron. Because the effects of these two factors on the magnitude of S probably are minor, we set $S = 6 \times 10^{-6} \text{ cm}^2$ as a first approximation.

The estimate for K , the deposition velocity, is derived from sections 2 and 6 in Mankin & Mayer (1982). The magnitude of K for a wide range of chemicals and adsorbent surfaces has been found to range from about 0.01 to 3 cm/sec (McMahon & Denison, 1970), and we set $K = 1 \text{ cm/sec}$ as a general estimate.