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Sensitivity of *Trichoplusia ni* (Hübner) pheromone receptor neurons: Relationships between neural thresholds and behavioral responses

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Summary. 1. Responses of *Trichoplusia ni* HS(A) receptor neurons were measured to determine the minimum detectable concentration (absolute threshold) and the minimum detectable increment (difference threshold) for the major sex pheromone component (*Z*)-7-dodecen-1-ol acetate (Z7-12:Ac). The absolute threshold was 1000-fold below the $\sim 10^{-11}$ *M* level of Z7-12:Ac at a calling female. The Weber fraction, i.e., the ratio of the difference threshold to the stimulus concentration, declined from ~ 0.8 to ~ 0.06 as the concentration rose from threshold to high intensities. Relatively smaller fluctuations were detected as the stimulus increased.

2. The HS(A) responses were interpreted in relation to behavior by considering an ideal observer as approximating the central nervous system (CNS). The ideal thresholds were 3–9-fold lower than the HS(A) thresholds.

3. The ideal absolute threshold of the *T. ni* CNS is comparable to observed behavioral thresholds for wing-flutter and taking flight. However, only a low percentage response occurs at threshold. Most males take flight at higher concentrations. Also, the ideal Weber fraction is lower than in most flight-tunnel bioassays. Yet, males respond to small fluctuations in orienting to pheromone plumes. These differences between moths and ideal observers may reflect inhibition at points in the CNS that control the flow of olfactory input.

Key words: Threshold – Olfaction – Insect – Lepidoptera – Noctuid

Introduction

Male moth sexual behavior is triggered primarily by pheromone-sensitive receptor neuron input to the central nervous system (CNS) (e.g., Light 1986; Getz and Chapman 1987; Christensen and Hildebrand 1987; Visser and De Jong 1988). This report examines the absolute and difference thresholds of Type HS(A) receptor neurons in *Trichoplusia ni* (Hübner). The HS(A) neurons detect the major sex pheromone component, (*Z*)-7-dodecen-1-ol acetate (Z7-12:Ac) (O'Connell et al. 1983; Grant and O'Connell 1986; Mayer and Mankin 1987; Mankin et al. 1987). Our objective was to interpret these thresholds relative to male *T. ni* behavioral responses in the literature.

A threshold is a minimum detectable absolute concentration or a minimum detectable concentration increment (Gesheider 1976; Dethier and Bowden 1984; Krueger 1989). The absolute threshold of a pheromone receptor neuron is the minimum concentration that evokes a mean frequency of action potentials (spikes) statistically different from the mean spontaneous activity. The neural difference threshold (DT), depicted in Fig. 1, is a minimum detectable change in concentration (C). The difference threshold is proportional to stimulus in-

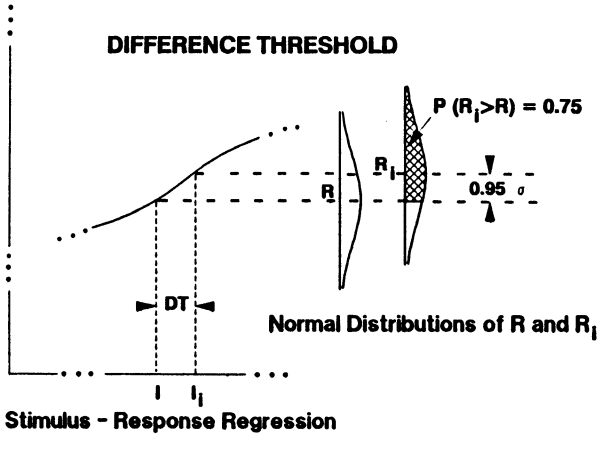

Fig. 1. Difference threshold (DT) as defined in statistical decision theory. Definitions: I, I_i = initial and incremented stimulus intensities, respectively; R, R_i = corresponding initial and incremented responses, respectively; *P*, the probability that $R_i > R$ (Criterion for DT is $P = 0.75$); σ = standard deviation of response

Abbreviations: *C* concentration; *CNS* central nervous system; *DT* difference threshold; *FSPG* female sex pheromone glands; *HS(A)* receptor neurons sensitive to Z7-12:Ac; *LDT* logarithmic difference threshold; *M* moles/liter; *T. ni* Trichoplusia ni; *W* weber fraction (=DT/C); *Z7-12:Ac* (Z)-7-dodecen-1-ol acetate

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tensity (Johnson 1980a, b). The Weber fraction is the relative sensitivity to intensity differences, $W = DT/C$. The Weber fraction decreases with increasing stimulus intensity (Johnson 1980). It is approximately constant near the middle of the response range (Gesheider 1976; Dethier and Bowdan 1984).

To interpret the neural thresholds in relation to behavior, we applied statistical decision theory to predict the response of an ideal observer (Johnson 1980a, b; Mankin and Mayer 1983a, b; Maes 1984). An ideal observer simultaneously combines all input from peripheral detectors. It makes decisions based on the minimum stimulus level discriminable from random noise. Comparisons between actual behavior and an ideal observer provide insight into CNS processes controlling behavior.

Materials and methods

Male and female *T. ni* pupae reared as in Guy et al. (1985) were segregated and held in 70–80% RH, 24–27 °C, on a 14:10 L:D cycle. Males were tested 2–3 days after eclosion. Except for pheromone glands, the stimuli were prepared from serial dilutions of Z7-12:Ac (>99% purity) in hexane. The glands were excised immediately before use from females 2–5 days after eclosion.

Action potentials were recorded extracellularly with tungsten electrodes. The recording electrode was inserted at the base of a sensillum, usually near the medio-distal margin of a subsegment. The reference electrode went into the lumen of a distal subsegment. A Grass P15® high-impedance preamplifier increased the signal 1000×. A Keithley Model 840® amplifier further increased the signal 10×. The amplified signal was ported to a 10-kHz A/D board on a PDP-11/23® microcomputer. User-written software stored the potentials on disk and classified the spikes elicited from each neuron in the sensillum (Mankin et al. 1987). A keystroke initiated a 10-s recording period comprising pre-stimulus, stimulus, and post-stimulus intervals of 2, 3, and 4 s, respectively.

The pheromone evaporated from the surface of a glass dispenser assembly (Mayer et al. 1987) which led into a delivery device similar to the one described by Grant et al. (1989). Until stimulus onset, the antenna rested in a stream of clean carrier air (either 1000 ml/min or 1200 ml/min). At stimulus onset, a computer-actuated valve switched a 200-ml/min pheromone stream into a chamber where it mixed with carrier air and exited to the antenna.

The Z7-12:Ac concentration passing over the antenna was calculated in units of moles/l (*M*) by dividing the dispenser emission rate (from Mayer et al. 1987) by the total mixture volume. Due to the shape of the dose-emission rate curve, a dosage increment had a proportionally greater effect on emission rate between 0.01 and 1 µg than between 1 and 10 µg doses.

In the initial part of the study we measured responses from 18 different sensilla of 10 different males to a hexane control and 5 doses of Z7-12:Ac. The doses increased in 0.5-log-unit steps from 0.01 µg to 1.0 µg. Stimuli were spaced about 5 min apart to reduce the effects of adaptation and contamination. Two or more replicates were done at all but the 1-µg dose. Testing ended after one stimulation at 1 µg if the post-stimulus response failed to return to the pre-stimulus levels within 5 min.

A second test series examined the responses to 0.1 and 10-µg dosages of Z7-12:Ac and 1 or 3 female sex pheromone glands, 1FSPG or 3FSPG, respectively. The post-stimulus response remained elevated at 10 µg. This dosage was presented only once to 6 sensilla on different antennae. Five sensilla on separate antennae received the 1FSPG stimulus (9 replications). Two sensilla on separate antennae had the 3FSPG stimulus (6 replications).

The spike records were analyzed separately by two different procedures. Initially, we averaged the responses from each neuron over the total 3-s stimulus period (time-averaged). Differences among means at different doses were evaluated by Duncan's Test (SAS Institute Inc. 1985).

In the second procedure (neuron-averaged analysis), we constructed peri-stimulus-time histograms (e.g., Gerstein et al. 1989; Rumbo 1981). Responses were averaged across all neurons tested at a given dose and time after the initiation of recording. Each 10-s record subdivided into one hundred 0.1-s bins. The number of spikes from all records obtained at a given dose were summed for each bin (Mankin et al. 1987). The number of spikes/bin divided by the number of records determined the mean counts/0.1 s.

Results

The receptor neuron responses depended on the Z7-12:Ac dosage, the time after stimulus initiation, and the neuron being tested. By contrast, the absolute and difference thresholds are defined as fixed concentrations independent of time or sampling procedure. We considered these effects by performing two analyses which dealt differently with time and sampling effects.

Analysis of responses in 3-s intervals (time averaged)

The initial analysis was performed on responses averaged over the 3-s stimulus interval (Table 1). This method neglects the temporal pattern of response which may be of considerable importance in CNS processing (Grant et al. 1989). If the CNS extracts information from the temporal pattern, a time-averaged threshold calculation

Table 1a) Analysis of variance of the mean spike frequency elicited during 3-s stimulus periods. Mean frequencies followed by the same letter are not significantly different at the $P = 0.05$ level by Duncan's test. **b)** Variance of spike frequency partitioned among effects of dose, neuron, and neuron * dose interaction (10-µg dose excluded).

Definitions: Conc, concentration of Z7-12:Ac in the stimulus plume; Dose, dispenser dosage of Z7-12:Ac in µg, except for the stimuli produced by 3 and 1 glands, 3FSPG and 1FSPG, respectively; Mean Freq (Sp/s), mean number of spikes elicited during the 3-s stimulus interval; *N*, number of replications at each dose

a)

Dose (µg)	Log Conc (M)	N	Mean Freq (Sp/s)
3FSPG	–	6	66.4a
1.0	−10.6	12	30.3b
1FSPG	–	9	24.1c
0.316	−11.6	44	14.1d
0.10	−12.7	49	7.0e
0.0316	−13.7	42	2.0f
0.01	−14.7	40	1.1f
Hexane	–	46	0.7f

b)

Source	DF	F Value	Pr>F
Dose	7	179.7	0.0001
Neuron	25	11.4	0.0001
Neuron * Dose	80	4.0	0.0001

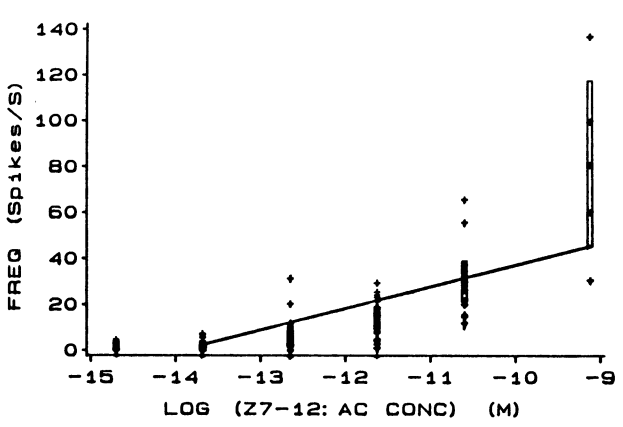

Fig. 2. Regression of mean spike frequency on Log(Z7-12:Ac concentration). Observations are designated by + (some observations overlap), and standard errors by open bars

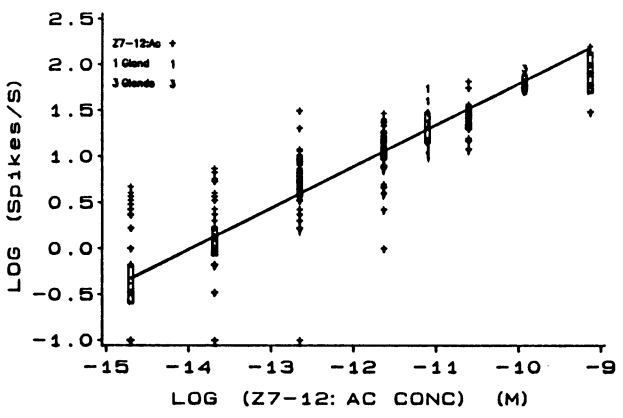

Fig. 3. Regression of mean Log(spike frequency) on Log(Z7-12:Ac concentration). Observations are designated by + (some observations overlap), and standard errors by open bars. Mean responses to 1 and 3 female glands are plotted where they lie on the concentration axis

probably overestimates the actual value. The analyses below suggest the time-averaged method overestimates the actual threshold at least 10-fold. In Table 1, the 0.1-μg dose elicited the lowest 3-s mean response that differs significantly from the hexane control. The 0.1-μg dose corresponds to a threshold concentration of $2 \cdot 10^{-13}$ *M*, based on the Mayer et al. (1987) dispenser calibration.

A regression of mean response on log-concentration is shown in Fig. 2. The standard errors were proportional to stimulus intensity. A power function transformation (Mankin and Mayer 1983a, b; Mayer et al. 1987; Laffort and Hoehn 1987) eliminated the dependence of the variance on concentration (Fig. 3). The power function (Eq. II, Table 2) produces a linear relationship between the logarithm of spike frequency and the logarithm of stimulus concentration:

$$\text{Log(Freq)} = \text{A} + \text{B Log(Concentration)}, \quad (1)$$

where A = 6.29 and B = 0.45 are regression constants. The slope, B, is of interest in the calculation of the difference threshold. Regressions on concentration also were calculated for mean responses in the first 0.5-s, first 1-s, and first 2-s of stimulation. The slope was independent of sampling duration, so those results are not presented.

Table 2. Summary of regression analyses performed on stimulus-response equations in text

Eqn. # in Table	Equation	Eqn. # in Text
I	Freq = A + B Log(Concentration)	–
II	Log(Freq) = A + B Log(Concentration)	(1)
III	Log(DT/Concentration) = Log(K) $-\beta$ Log(Concentration)	(8)

Definitions: A, B, K, and β, regression constants; DT, difference threshold; r^2, coefficient of determination, Root MSE, square root of mean square error ($\sigma_{1s} = \sigma_1$ in Eqs. 3 and 5)

Eqn. #	Regression Constant	Estimate	Standard Error	F	r^2	Root MSE
I	A	131.64	9.353	169.6	0.47	14.184
	B	9.45	0.725			
II	A	6.29	0.309	353.58	0.65	0.468
	B	0.45	0.024			
III	Log(K)	−3.08	0.40	40.5	0.90	0.151
	β	0.21	0.033			

The responses to 1 and 3 pheromone glands (1FSPG and 3FSPG, respectively) were plotted in Fig. 3 where their log-means fell on the regression line. The mean 3FSPG response occurs at a concentration of $1.6 \cdot 10^{-10}$ *M*, or $0.53 \cdot 10^{-10}$ *M* per gland. The mean 1FSPG response occurs at $0.13 \cdot 10^{-10}$ *M*. The location of the 1FSPG and 3FSPG responses near −10.9 and −9.8 on the regression line is in good agreement with previously published *T. ni* pheromone gland emission rates. The emission rates in Sower et al. (1971), Bjostad et al. (1980), and Baker et al. (1981): $3.1 \cdot 10^{-5}$ μmol/min, $5.3 \cdot 10^{-5}$–$9.7 \cdot 10^{-5}$ μmol/min, and $1.06 \cdot 10^{-5}$ μmol/min, respectively, divided by the delivery device flow of 1400 ml/min, yield concentrations between $0.076 \cdot 10^{-10}$ *M* and $0.69 \cdot 10^{-10}$ *M*. These points lie at −11.1 and −10.2 on the regression line.

Analysis of responses in 0.1-s intervals (neuron-averaged)

Peri-stimulus-time histograms (Fig. 4) were computed for responses averaged over all neurons tested at a given dose. The pattern in Fig. 4 is what an ideal observer obtains by summing contributions from all detectors simultaneously. A similar pattern pertains if the HS(A) neurons converge in the deutocerebrum, as has been found for other insects (e.g., Boeckh and Boeckh 1979; Christensen and Hildebrand 1987).

Analysis of variance (Table 3) indicates the lowest statistically significant response occurs at 0.0316 μg. The Z7-12:Ac concentration at this dosage (Mayer et al.

Table 3. Analysis of HS(A) responses integrated in 0.1-s intervals (bins) (see Methods and Fig. 4). Mean frequency and standard deviation, σ_m, (Cols. 3–4) were calculated from counts/s (=10 counts/0.1 s) in the 5 bins beginning at t=4.0 s. Log mean frequency and log standard deviation, σ_{lm}, (Cols. 5–6) were calculated from Log(counts/s) (arithmetic and geometric [log] means are not necessarily equal). Means in Cols. 3 and 5 followed by the same letter are not significantly different at $P=0.05$ by Duncan's Test. The difference threshold (DT) in Column 7 was computed from Eqs. 5–7 using σ_{lm} in Col. 6 and the concentrations in Col. 2. The Weber fraction is DT/C (Col. 7/Col. 2)

1 Dose (μg)	2 Conc. (C) (10^{-12} M)	3 Mean Freq (Counts/s)	4 σ_m	5 Mean Log Freq	6 σ_{lm}	7 Diff Thrsh (DT) (10^{-12} M)	8 Weber Fraction (DT/C)
10.0	794.0	112.4a	3.05	2.05a	0.012	47.7	0.06
1.0	25.1	33.0b	1.87	1.52b	0.025	3.25	0.13
0.316	2.51	16.6c	2.51	1.22c	0.067	0.967	0.39
0.1	0.199	7.2d	0.84	0.85d	0.052	0.0574	0.29
0.0316	0.0199	3.2e	0.84	0.49e	0.124	0.0165	0.83
0.01	0.00199	0.8f	0.45	−0.06f	0.134	0.00165	0.83
Hexane	–	0.6f	0.55	−0.12f	0.164	–	–

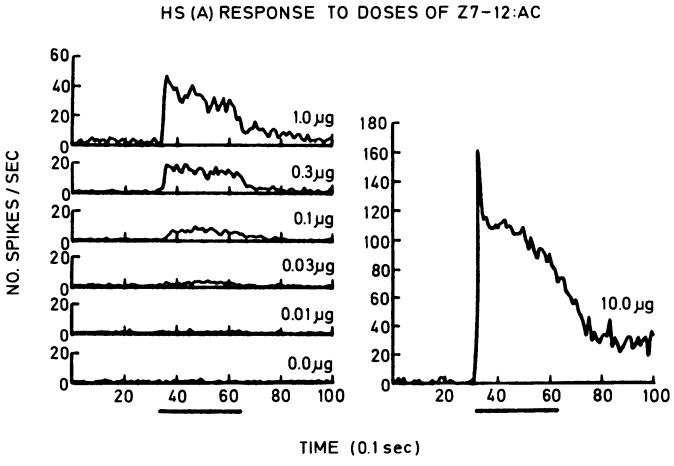

Fig. 4. Mean HS(A) responses integrated over 0.1-s intervals. The 3-s stimulus period is indicated by the solid bar

1987) is approximately $2\cdot10^{-14}$ M, 10-fold below the time-averaged estimate of $2\cdot10^{-13}$ M. The true threshold, however, may lie even below 10^{-14} M. The difference threshold analysis below suggests the absolute neural threshold is nearer 10^{-15} M. Even so, the HS(A) absolute threshold probably lies above the levels of 10^{-17} M–10^{-18} M reported for other neurons in Kaissling (1987), Mayer and Mankin (1990), and Mankin (1991).

Difference threshold

The difference threshold is relatively simpler to interpret for the neural response than for behavior. In Fig. 1, the neural threshold is the minimum stimulus increment,

$$DT = C_i - C, \tag{2a}$$

satisfying a criterion that the response, R_i, to the incremented stimulus, C_i, has a 75% probability of exceeding the response, R, to the initial stimulus, C. The probability level of $P=0.75$ is achieved when

$$R_i - R = 0.95\,\sigma, \tag{2b}$$

where σ is the standard deviation (Johnson 1980b). Comparisons among thresholds usually are expressed in terms of the Weber fraction,

$$W = DT/C. \tag{2c}$$

The problem in defining a behavioral correlate of Eqs. 2a–c is that initial detection often is more behaviorally relevant than a simple change in the stimulus level. Bioassays must be designed carefully to give behavioral relevance to small changes in stimulus intensity. Otherwise, the insect may detect but not respond overtly to stimulus differences.

An estimate for DT is determined from the neural records through Eq. 1. The responses, R_i and R, are expressed as logarithms of spike frequency. Thus, Eq. 2b can be expressed as

$$\mathrm{Log}(\mathrm{Freq}_i) - \mathrm{Log}(\mathrm{Freq}) = 0.95\,\sigma_l, \tag{3}$$

where Freq_i and Freq are the spike frequencies stimulated by I_i and I, respectively, and σ_l is the standard deviation of Log(Freq). We define also a logarithmic difference threshold (LDT) similar to Eq. 2a:

$$LDT = \mathrm{Log}(C_i) - \mathrm{Log}(C). \tag{4}$$

The antilog of the LDT is C_i/C, the minimum discriminable ratio. The relationship between DT and LDT follows from Eq. 2a:

$$DT = C_i - C = C\cdot(C_i/C - 1) = C\cdot(10^{LDT} - 1). \tag{5}$$

From Eqs. 1 and 3:

$$LDT = 0.95\,\sigma_l/B, \tag{6}$$

so that

$$DT = C\cdot(10^{0.95\sigma l/B} - 1) \tag{7}$$

Table 4. Behavioral difference thresholds to sex pheromone (DT). The thresholds are expressed as the upper limit of the Weber fraction, DT/C. In most cases, smaller intensity differences were not tested

Insect	Chemical	Reference	Upper Limit W = DT/C
T. ni	Z7-12:Ac	Sharma et al. (1971) Mayer (1973)	2.0 4.0
B. mori	ZE-10, 12-16:OH	Kramer (1975)	0.7
G. molesta	Z8-12:Ac + E8-12:Ac + Z8-12:OH	Kuenen and Baker (1982)	2.0
C. fumiferana	E11-14:Al + Z11-14:Al	Sanders (1982)	9.0
P. interpunctella	ZE-9,12-14:Ac + ZE-9,12-14:OH	Mankin et al. (1983)	1.9
L. dispar	(+)-*cis*-7,8-epoxy-2-methyl-octadecane	Hagaman and Cardé (1984)	9.0
D. brevicomis	*exo*-brevicomin + frontalin	Byers (1988)	9.0
I. paraconfusus	ipsenol + ipsdienol + *cis*-verbenol	Akers (1989)	0.3

where B is the regression slope in Eq. 1 (=0.45 in Table 2).

The estimate for the HS(A) difference threshold in Eqs. 7 depends on the method used to calculate σ_1. The root mean square error of Eq. II (Table 2), $\sigma_{1s}=0.468$, provides a pooled estimate for the standard deviation, from which LDT = 0.988 in Eq. 6. The antilog is the difference ratio, $C_i/C = 9.73$, so that W = 8.73 in Eq. 2c. This is unrealistically high from a behavioral perspective. Considerably lower Weber fractions appear in the literature (Table 4, Gesheider 1976; Kramer 1976; Bell and Tobin 1982). Also, this method provides only a single estimate of DT, which varies with stimulus concentration (Johnson 1980a, b; Dethier and Bowdan 1984; Krueger 1989).

To estimate a difference threshold that incorporates effects of concentration, we calculated a standard deviation from the bin-count variance in Fig. 4. Table 3 lists values for the standard deviation of Frequency (σ_m in Col. 4) and Log Frequency (σ_{1m} in Col. 6) at each dispenser dosage. In Fig. 5, the difference thresholds calculated from the standard deviation of Log Frequency (DT in Col. 7) are expressed as Weber fractions and plotted against Log C. The Weber fraction decreases with increasing Z7-12:Ac intensity from 0.83 at $2\cdot10^{-14}$ *M* to 0.06 at $7.9\cdot10^{-9}$ *M*. The decrease is predicted by signal detection theory (Johnson 1980b):

$$W = KC^{-\beta}, \tag{8}$$

where $\log K = -3.08$ ($K = 8.32\times10^{-3}$) and $\beta = 0.21$ are regression constants (Eq. III in Table 2). Theoretically, $\beta = B/2$ (Johnson 1980b), a result which holds in Table 2.

In Fig. 6, we compared the two analyses by plotting the thresholds as steps on the HS(A) regression line. Each step was 0.988 log units for the time-averaged, σ_{1s}-based (dashed) line. Steps based on the neuron-averaged, σ_{1m}-based (solid) line were calculated from Eq. III, Table 2. The former represents a minimum of stimulus level information the CNS can extract from these neurons, and the latter, what an ideal observer extracts.

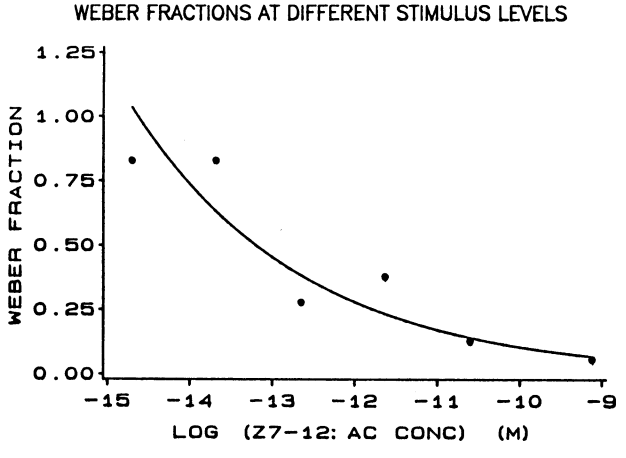

Fig. 5. Regression of Weber fraction (DT/C) on Log Z7-12:Ac concentration (Eq. III in Table 2). The closed circles are Weber fractions computed from Table 3

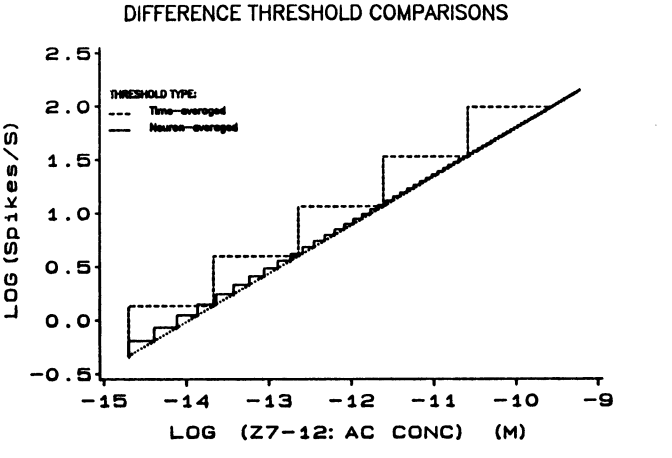

Fig. 6. Comparisons of difference thresholds computed from variance of 3-s mean responses (dashed line), and 0.1-s mean responses (solid line). Five concentration levels can be distinguished based on the former estimate, and 134 levels based on the latter estimate

In Fig. 6, the threshold differences extend several steps past the absolute threshold. The presence of these steps suggests there is a discrepancy between the absolute and difference threshold analyses. If the difference analysis is correct, a statistically significant difference exists for responses to the control, $10^{-14.7}M$ and $10^{-13.7}M$. Part of this discrepancy is due to the difference in the significance level ($P=0.95$ for the absolute threshold; $P=0.75$ for the difference threshold). Also, the difference analysis may have a greater resolving power than the absolute analysis. If so, the true absolute threshold probably is somewhat lower than the $10^{-13.7}M$ estimate.

Discussion

Although the HS(A) thresholds are of interest from a neurophysiological perspective, our main concern is their effect on male *T. ni* behavior. To explore this relationship, we began by examining the difference between the HS(A) neural thresholds and *T. ni* behavioral thresholds to Z7-12:Ac. There were some differences in both cases. However, we found better agreement between the absolute thresholds than between the difference thresholds. The reasons for a relatively close agreement in one case and a large discrepancy in the other are the major focus of this discussion.

Behavioral responses and the HS(A) absolute threshold

First we compared the HS(A) absolute threshold with the previously published *T. ni* thresholds. Sower et al. (1971) measured the threshold for wing-flutter to Z7-12:Ac as $10^{-17}M$. Mayer (1973) (see also Mayer et al. 1987) measured the threshold for taking flight as $10^{-15}M$. Both measurements lie somewhat above the absolute detection threshold in the CNS, considering the central processing necessary to elicit wing flutter and flight. However, even a conservative estimate of $10^{-15}M$ lies well below the threshold of $2\cdot10^{-14}M$ determined for the HS(A) neurons in this report.

We interpret the differences between these thresholds to be due, at least in part, to a reduction in the variance of the signal transmitted to the CNS. The expected reduction in variance is estimated from the variance for an ideal observer that receives the same input as the insect CNS (Johnson 1980a, b; Mankin 1983a, b). The ideal observer responds whenever the signal intensity rises significantly above the detector noise level. When a signal comes from multiple detectors, the combined signal has a smaller variance than the signal from a single detector. The reduction in variance, R_σ, is proportional to the square root of the degrees of freedom [number of detectors -1] (Mankin and Mayer 1983a, b and references therein; Meng et al. 1989). A reduction in variance also occurs in multiple sampling of one detector (Gerstein et al. 1989). The response variance in the approximately 40 recordings per dose in Fig. 4, for example, is less than from two records by a factor of $R_\sigma \sim 39^{0.5} = 6.24$.

The magnitude of R_σ in the *T. ni* CNS is uncertain because neither the number of converging neurons, nor the number of loci onto which they converge are known precisely. We estimate the fraction of the 5400 Type I sensilla (Mayer et al. 1981) which contain HS(A) neurons is about 60%, or 3000 HS(A) neurons per male antenna. Based on morphological studies (Homberg et al. 1988) and recordings from the CNS of other insects (Boeckh and Boeckh 1979; Olberg 1983), these neurons converge onto at least one and probably no more than 30 loci.

The greatest reduction in variance occurs for an ideal observer that sums input from all detectors simultaneously. This is the case where all HS(A) receptor neurons converge at a single locus, or $R_\sigma \sim 2999^{0.5}/6.24 = 8.8 \sim 9$. (We divided by 6.24 because the variance already was reduced by this factor in averaging across $\sim$40 samples.) If only 1/10th, or 300 HS(A) neurons converge at a single locus, then $R_\sigma \sim 299^{0.5}/6.24 \sim 3$.

Using these estimates, the absolute threshold at a point of convergence lies between $2\cdot10^{-14}M$ and $2\cdot10^{-15}M$, perhaps near $7\cdot10^{-15}M$. This estimate still lies above the wing-flutter threshold estimate by Sower et al. (1971), but it is close to the $10^{-15}M$ threshold for taking flight obtained by Mayer (1973). Male *T. ni* that respond at either level thus approximate ideal observers.

Pheromone threshold studies usually focus on the sensitive ideal observer males. However, the percentage of male *T. ni* taking flight typically is low until the pheromone concentration exceeds threshold levels by $\sim$2 orders of magnitude (e.g., Mayer 1973; Landolt and Heath 1987). The majority of males thus do not respond as ideal observers. The failure to take flight at 10^{-13}–$10^{-12}M$ probably is not due to lack of sensitivity. The neural responses in Figs. 2–3 indicate that virtually all males can detect Z7-12:Ac easily at these levels. A similar discrepancy between real insects and ideal observers appears below when we consider the behavioral effects of small changes in stimulus intensity.

Behavioral responses and the HS(A) Weber fraction

Few assays of pheromone-elicited behavior have measured a behavioral difference threshold directly. However, there are several reports where percentage responses in a bioassay were compared at different pheromone intensities. Weber fractions estimated from these reports are presented in Table 4. The estimates are based on the smallest increments in intensity that elicited significant changes in behavioral response. Also, none of these bioassays tests the possibility that behavioral discrimination of stimulus intensity improves as the stimulus level increases.

All of the Weber fractions in Table 4 lie above those expected from the neural difference thresholds in Table 3. In most cases, the values in Table 4 were upper limits for the true Weber fractions because the bioassays were done with large increments in concentration. Even taking this into account, the discrepancy is considerable.

The discrepancy in the Weber fractions is even greater for an ideal observer. The difference threshold for an ideal observer is 9-fold lower than the HS(A) neural threshold. Similarly, the difference threshold at a point of convergence in the CNS is expected to be 3-fold to 9-fold lower than the HS(A) threshold. If a male *T. ni* behaves as an ideal observer, it can distinguish fractional changes in Z7-12:Ac concentration (Weber fractions) of $\sim 0.06/9 = 0.007$ to $\sim 0.06/3 = 0.02$ at concentrations 100-fold above those emitted by a female (10-μg dose at $10^{-9.1}$ *M* in Figs. 3 and 6). It is more difficult to distinguish changes in stimulus intensity near the absolute HS(A) threshold. However, the male still should be able to distinguish fractional changes of $\sim 0.8/9 \sim 0.09$ to $\sim 0.8/3 \sim 0.28$.

In this case, part of the discrepancy between predicted and observed difference thresholds is due to the type of bioassay rather than to lack of behavioral capability. A learning or choice bioassay designed specifically to test discrimination ability probably would yield Weber fractions much closer to those of an ideal observer (e.g., Kramer 1976; Shimada et al. 1987). Certainly, there are occasions when discrimination of small changes in intensity is important, such as orientation in a pheromone plume (e.g., Kuenen and Baker 1982; Akers 1989).

Some evidence that moths have a high sensitivity to small differences comes from studies of pheromone blend ratio. When each component of a pheromone blend is detected by a separate type of receptor neuron, the discrimination of blend ratio is similar to the discrimination of blend intensity (Getz and Chapman 1987; Den Otter 1977; Visser and De Jong 1988). Both situations involve comparisons between two signals, each of which is subject to random fluctuations. The 0.007–0.28 Weber fractions estimated above are similar to the minimum detectable differences in pheromone blend ratio reported in pheromone trapping studies (e.g., Baker et al. 1976; Baker and Cardé 1979; Landolt et al. 1986) and in laboratory bioassays (e.g., Linn and Roelofs 1983). Thus, both neural and behavioral data suggest that male *T. ni* can detect smaller differences in Z7-12:Ac intensity than reported in bioassays of percentage wing flutter or taking flight.

Interplay between excitatory and inhibitory central processes

The preceding comparisons of neural and behavioral thresholds indicate that male *T. ni* usually are less responsive to Z7-12:Ac than an ideal observer. A possible explanation for this lack of responsiveness is inhibition at loci in the CNS that control the flow of olfactory input.

In support of such a hypothesis, we note that other factors besides the concentration of Z7-12:Ac also can affect the percentage of males taking flight. These factors include the presence or absence of other pheromone blend components (Linn et al. 1987), light intensity (Shorey 1966), time of day (Shorey and Gaston 1964), temperature (Cardé and Charlton 1984; Linn et al. 1988), and high-frequency acoustical stimulation (Baker and Cardé 1978). With extremely inhibitory conditions such as daylight, male *T. ni* fail to take flight even at high concentrations of Z7-12:Ac.

Under such a hypothesis, bioassays of pheromone-elicited behavior measure the effects of an interplay between excitatory and inhibitory CNS processes. If the bioassay conditions are ideal and central inhibition is low, males will take flight at pheromone concentrations approaching the HS(A) absolute threshold. Otherwise, the concentration must rise 2–3 orders of magnitude above the absolute neural threshold to overcome central inhibition.

A similar argument holds for the Weber fraction in a bioassay of pheromone-elicited behavior. If central inhibition is low, almost all males take flight near the absolute threshold. The Weber fraction for behavior approaches that of an ideal observer. Otherwise, the concentration must rise to overcome both random neural noise and central inhibition. This results in a higher difference threshold than predicted for an ideal observer which must overcome only random neural noise. High levels of central inhibition also can mask the reduction that occurs in the HS(A) Weber fraction when the pheromone intensity increases above threshold (Fig. 6). Consequently, there may be no observable correlate of Eq. 8 in the behavioral response.

Although it reduces behavioral responsiveness, strict central inhibitory control over pheromone-elicited sexual behavior appears to be evolutionarily advantageous. When species share components of a pheromone blend, central inhibition of responses to inappropriate blends can evolve to improve species isolation. Yet, the ability to detect small fluctuations can facilitate orientation up the pheromone plume once mate-seeking behavior is initiated. Other central inhibitory processes can restrict the expression of sexual behavior to appropriate photoperiod and environmental conditions.

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