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Review article

Olfactory response characteristics and tuning structure of placodes in the honey bee *Apis mellifera* L

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Summary — Little is known about how honey bee sensory neurons detect ordinary (nonpheromonal) odors because: i) simultaneous response of relatively large numbers of olfactory neurons in a honey bee olfactory placode sensillum makes it difficult to interpret extracellular recordings from these sensilla; and ii) the majority of insect olfactory studies focus on questions relating to the detection of sex and aggregation pheromones rather than ordinary odors. Here we discuss the problem of ordinary odor detection in a general context and then review what is know about the response of honey bee sensory neurons to ordinary odors. We also present new analyses of data recently obtained from extracellular recordings of the response of honey bee worker placode sensilla to single and binary combinations of various odorants.

sensory neuron / olfactory coding / electrophysiology / placode / olfaction

INTRODUCTION

The study of how olfactory sensory neurons respond to stimuli presents a special challenge in honey bees compared with a number of species of moths (*eg* Kaissling and Priesner, 1970; van Der Pers and Den Otter, 1978; Grant and O'Connell, 1986; van Der Pers and Löfstedt, 1986; Kaissling *et al,* 1989; Akers and O'Connell, 1991), beetles (Boeckh, 1962; Tømmeras *et al,* 1984; De Jong and Visser, 1988), and cockroaches (Sass, 1978; Selzer, 1984; Fujimura *et al,* 1991), among others. In these latter organisms, 1–3 sensory neurons are located in hair-, peg-, or pit-like sensilla and the responses of individual sensory neurons are usually easily identified in extracellular single-sensillum recordings (fig 1). In honeybees, however, 5–35 sensory neurons (Schneider and Steinbrecht, 1968) are located under oval plates called placodes. The activity of several sensory neurons, especially in workers as opposed to drones, is evident in recordings from these placodes

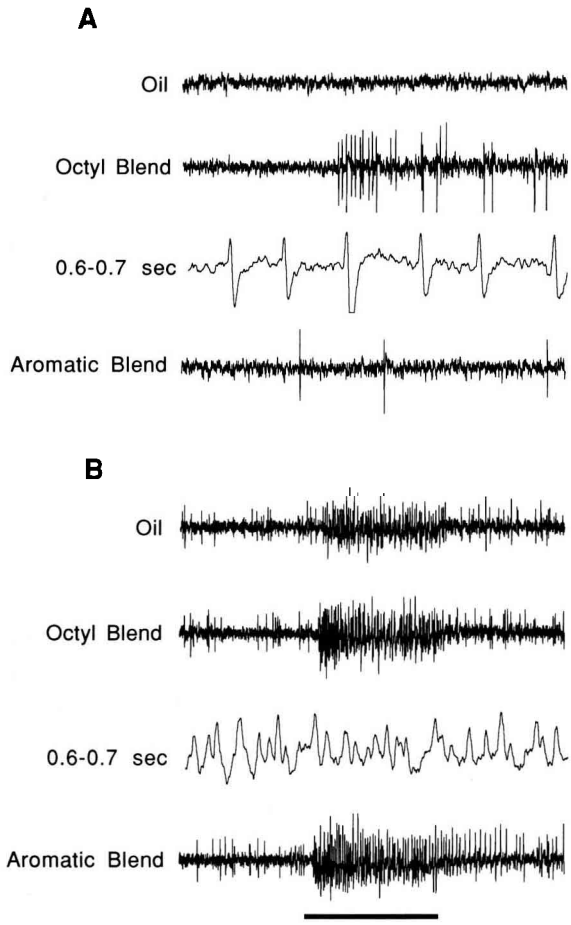

Fig 1. Extracellular recordings, made over a 1.5-s interval (the bar represents the 0.5-s interval over which the stimulus was applied) under identical conditions, of responses of sensory cells in: A) an antennal peg (basiconic sensillum) of the American cockroach, *Periplaneta americana*; and B) an antennal pore plate (placode sensillum) of a honey bee worker, *Apis mellifera* to a mineral oil control and to a blend of octyl and a blend of aromatic odorants (see tables II and III) for lists of blend components) at 15 μg concentrations per μl mineral oil. The expanded traces are the 0.6–0.7-s intervals of the responses the corresponding octyl blends. Note that the honey bee placode responds to the oil control, which is probably a response to change in humidity (Akers and Getz, 1992).

(fig 1), and the firing rate of individual sensory neurons can only be extracted using computationally-based spike-sorting techniques.

Vareschi (1971) tried to overcome the difficulties in interpreting extracellular recordings from honey bee sensilla by: i) working primarily with drones rather than

workers; and ii) using a so-called competitive adaptation technique (Boeckh *et al*, 1965). This technique involves 2 odors, the first of which Vareschi applied as a 600-ms conditioning stimulus followed 200 ms later by a 600-ms test stimulus. This dual stimulus application was repeated for increasing concentrations of the conditioning stimulus, the concentration of the test stimulus remaining constant.

If the response to the test stimulus is significantly reduced at high concentrations compared with low concentrations of the conditioning stimulus, the 2 stimuli are assumed to excite the same receptor neuron. This assumption can be validated further, as in Vareschi (1971), by reversing the roles of the 2 stimuli to ensure that the effects are symmetrical.

Competitive and cross-adaptation techniques discussed below can be used to identify whether placodes responsive to both stimuli contain individual neurons that are primarily responsive to one stimulus or the other. However, the multiple innervation of placodes confounds the results. For example, a placode may well contain one cell sensitive to odor A, a second to odor B, and a third to both odors A and B. The adaptation of the response of this third cell to odor B because of the increasing strength of application of the conditioning odor A could be masked by the continuing response of the second cell; and the same problem would occur due to the presence of the first cell if the roles of both odors were reversed. Further, the competitive adaptation technique ignores details of the adaptation processes (*eg*, whether recovery rates are different for different odorants, whether different odorants compete for the same or different membrane receptor proteins, and whether the different receptor proteins on the same membrane use the same or different second messenger systems). Finally, from the data presented here, it is apparent that sensory neurons in the same placode inhibit (or pace) one another so that high concentrations of an odorant may inhibit the firing of cells that are not individually inhibited by that odorant.

Despite these difficulties, the competitive adaptation technique provides one method for obtaining some insight into the response characteristics of olfactory sensory neurons in honey bee placodes. Below we review Vareschi's findings, which are based primarily on recordings made from drones rather than workers.

A second approach to obtaining the response characteristics of olfactory sensory neurons in honey bee placodes is to make extracellular recordings of the response of single placodes to a variety of stimuli and then to try and identify the responses of individual neurons in this trace using spike shape (fig 1). Of course, a third approach is to record directly from single cells, but this approach presents challenges that as yet have not been fully overcome.

We have made extensive extracellular recordings from the placodes of worker honey bees. The details of the methods are reported elsewhere (Akers and Getz, 1992, 1993). In summary, using tungsten electrodes, we recorded from the antennal placodes of whole organisms fixed in a harness. A 210-ml/min airstream of odorless air (purge) and a 60-ml/min airstream of odor-laden air (the stimulus) were aligned facing each other on either side of the antenna. This ensured that only odorless air flowed over the antenna, except during actual stimulation periods when the purge was switched off.

The stimuli were delivered for 0.5 s. Recording commenced 0.5 s before the onset of a stimulus and continued for 0.5 s after completion of a stimulus (fig 1). We prepared stimuli by dissolving odorants and mixtures of odorants (measured in μg) in 1 μl mineral oil, which was then applied to

a 7 x 35-mm rectangle of filter paper fitted into a cartridge through which a stream of air was blown to deliver the stimulus. In some cases the stimulus was a single odorant and in others a binary mixture or blend of odorants.

DATA ANALYSIS

The delivery of each stimulus was controlled by a microcomputer that employed solenoid valves to switch the purge and stimulus airstreams on and off. The same computer was used to capture the electrophysiological response (1.5-s recording sequence described above), using an analog-to-digital data acquisition card. Since the sampling rate was 10 000 Hz, each 3–4-ms spike was characterized by 30–40 points. This allowed us to identify the peak of each spike to an accuracy of 0.05 ms. From this record we categorized spikes according to shape (primarily height, but also width), using the software package SAPID Tools (Smith *et al,* 1990). In analyzing the data, one can consider either the total number of spikes over a selected interval of time or the number in each shape category over the same interval of time. One can also calculate the size of all the interspike intervals for all identified spikes (*ie* those spikes lying above a preselected noise threshold) in the trace or only those spikes belonging to a specific size class. In all spike trains that we analyzed, we identified 3–4 classes of spikes. Although we cannot be sure that each of these classes represents the response of an individual sensory neuron, from the results presented below it appears that the subplacode response units represented by each spike class provide information and insight that cannot be obtained from the unsorted placode data.

Much of the data we present below is based on the net number of spikes (*cf* fig 8) occurring over the interval 0.5–1.0 s (*ie* stimulus duration, although one of our data sets represents neuronal activity over 0.55–1.0 s). This number is calculated by subtracting the number of spikes obtained using a control stimulus (1 μl pure mineral oil – see fig 1) from the number of spikes obtained using a particular odorant stimulus. For the ith stimulus and the jth response unit (whole placode or subplacode, whichever is applicable) let this number be represented by r_{ij}. Then, if we evaluate the response of the unit j to a set S of n stimuli, ω_i, $i = 1, \ldots, n$, we obtain a net response vector $r_j = (r_{1j},\ldots,r_{nj})'$ (Akers and Getz, 1992) with respect to S (fig 2). The Euclidean norm of this vector, the constant

$$\| r_j \| = \sqrt{\sum_{i=1}^{n} r_{ij}^2}, \qquad [1]$$

is a measure of the overall sensitivity of the response units to the stimuli in question; and the direction of this vector, represented by the normalized vector

$$z_j = r_j / \| r_j \| \qquad [2]$$

(*ie*, each element z_{ij} of the vector r_j is the element r_{ij} divided by the constant $\| r_j \|$ and r_j describes a point on the surface of the unit n-dimensional sphere), characterizes the tuning of this response unit with respect to the stimulus set S.

Once the tuning characteristics of a sample of m placodes has been evaluated with respect to a stimulus set S by generating the m vectors z_j, $j = 1,\ldots, m$, then tuning categories can be formed using various forms of principal components and cluster analyses (Den Otter *et al,* 1980; Derby and Ache, 1984; Akers and Getz, 1992). Further, the geometric interpretation of the vectors z_j suggests that the tuning distance between any 2 response units j and k can be characterized by the angle θ between

these 2 vectors (Getz and Chapman, 1987), which is obtained by solving the equation:

$$\cos\theta_{jk} = \sum_{l=1}^{n} z_{jl}\, z_{kl} \qquad [3]$$

From equations [1] and [2] it follows that identically tuned units are given by $\cos\theta_{jk} = 1$ ($\Rightarrow$ $\theta_{jk} = 0$). Orthogonally tuned units are defined by $\cos\theta_{jk} = 0$ ($\Rightarrow$ $\theta_{jk} = \pi/2$) and oppositely tuned units by $\cos\theta_{jk} = -1$ ($\Rightarrow$ $\theta_{jk} = \pi$).

Response units can be considered as specialists or generalists respectively depending on whether their tuning vectors point in the general direction of one of the *n*-axes (the response to each stimulus in the stimulus set *S* defines an axis) or towards the centers of one of the *n* quadrants of the tuning space discussed above. The degree of specialization (*ie*, the extent to which the tuning vector aligns itself with one of the axes) can be quantified using, for example, the information theory measure *H* defined as follows (*cf* Girardot and Derby, 1990, or Smith and Travers, 1979):

$$H_j = -K \sum_{l=1}^{n} p_{ij} \log p_{ij}, \qquad [4]$$

where *K* is a suitable scaling constant, which could be set to $K = 1/\ln n$ to ensure $0 \leq H_j \leq 1$, and

$$p_{ij} = \frac{|\, z_{ij} \,|}{\sum_{l=1}^{n} |\, z_{lj} \,|} \qquad [5]$$

is the proportional normalized response of the unit to the *i*th odorant. Now, if z_j is aligned along the *i*th axis, then $z_{ij} = 1$, $z_{lj} = 0$, for all $l \neq i$ and equations [4] and [5] imply $H_j = 0$. On the other hand, if z_j points into the center of one of the *n* quadrants (*ie*, the magnitude of the response – irrespective of whether it is excitation or inhibition – to all stimuli S_i is the same), then for $i = 1,\ldots n$, $|\, z_{ij} \,| = 1/\sqrt{n}$ and equations [4] and [5] imply $H_j = 1$. Note, however, by considering only the absolute value of z_{ij} in definition [5], we do not distinguish informationally between excitatory and inhibitory responses with respect to the background control. To do so would require at least a 2-dimensional measure.

The above discussion ignores the temporal structure of the response over the stimulation interval. This structure has at least 2 facets: i) its phasic-tonic character; and ii) the nature of the distribution of the intervals between successive spikes. The phasic–tonic nature of the responses depends on both the intensity and the length of the stimulus and contains information relating to adaptation during stimulation and recovery after stimulation of the neuron. We will not focus on these characteristics here.

An analysis of the distribution of interspike intervals can be used to shed some light on questions relating to the number of cells contributing to the response of a subplacode unit and whether the superimposed response of several subplacode units indicates independent or organized firings of these units at the whole placode level (Akers and Getz, 1992). Specifically, for any type of response unit firing purely at random, the spike interval statistics should follow a Poisson distribution (Sachs, 1982): a Poisson distribution has a coefficient of variation (CV) equal to 100 where, for any distribution with mean μ and variance $\sigma 2$,

$$CV = 100\,\frac{\sigma}{\mu}$$

We should expect the distribution of interspike intervals of a sensory neuron to

be more regular then random – its CV should be < 100 – because spikes are prevented from being arbitrarily close together due to the refractory periods required for a neuron to recover between firings. Further, once the refractory period is over, if firing rates are then random we should expect the CV of sensory cells to increase towards 100 as mean-interspecific-intervals (MII) go up or, equivalently, mean firing rates decline. On the other hand, if interspike intervals are derived from the random superposition of several independently firing units, each representing a non-Poisson renewal process, then the MII of the combined spike train, although less than the MII of the individual placode trains approaches a Poisson distribution (Feller, 1966, ch XI.3): that is, the MII statistics for the combined spike train of a placode should increase towards 100 if the subplacode units are firing independently of one another.

VARESCHI'S CATEGORIES

Vareschi's (1971) data pertain primarily to recordings made from placodes on the antennae of honey bee drones. He categorized sensory neurons into a number of different classes based on the adaptive response of placodes, using the method described above, to 79 different odorants. Vareschi's study was particularly comprehensive from an odor stimulus point of view but, lacking modern techniques for digitizing data, was superficial from a spike analysis point of view.

Vareschi (1971) identified a minimum of 7 classes of cells, where each cell within a class reacts to a spectrum of odorants, but between classes the spectra are quite distinct. For example, cells in Vareschi's Class V react to citral, geraniol, citronellol, nerol and eugenol, while cells in Class VI react to linalool and limonene. Class VI has the unique property of containing some cells that were excited by stimuli and others that were inhibited by stimuli. The cells in all the other classes were indicated as only being excited by stimuli. Vareschi's Class I cells appear to respond to the greatest number of odorants among those tested, including a number of different fatty acids. Oddly enough, Vareschi listed the Class I cells as sensitive to 2-hexenal and 2-hexenol, but not to any of the other aldehydes or alcohols tested.

We decided to test Vareschi's categorization scheme by recording directly from placodes, as described above. In particular, we evaluated the response of 70 placodes to 10 μg/μl concentrations of 6 odorants: 2 odorants from Class I – undecanoic *(ud)* and dodecanoic acids *(dd)*, 2 from Class V – citral *(ct)* and geraniol *(gr)*, and 2 from Class VI – limonene *(li)* and linalool *(ll)*. We expected, based on Vareschi's results, that neurons responding strongly to *ud*, for example, would be much more likely to have a strong response to *dd* than to any of the other 4 odorants. A correlation matrix analysis of the tuning vector data obtained, first, for the placodes as a whole and, second, for the subplacode response units revealed a much more complicated situation than the classification scheme proposed by Vareschi (table I). At the whole placode level, for example, *ud* and *dd* showed stronger correlations to *li* (0.51 and 0.63 respectively) than to each other (0.42). Further, *ct* and *gr* were negatively correlated (–0.25). At the subplacode level, Vareschi's category structure did not emerge either, with *ud* showing a stronger correlation to *li* (0.40) and *dd* showing a stronger correlation to *ct* (0.39) than to each other (0.35). Although these differences are small and unlikely to be significant, they bring into question Vareschi's classification scheme.

The subplacode response units exhibited considerable variability in tuning char-

acteristics. A cluster analysis of subplacode tuning types (Akers and Getz, 1992) revealed that some subplacode units responded to almost all of the 6 odors (fig 2, cluster 4), some were inhibited by almost all 6 odorants (fig 2, cluster 5), some responded predominantly to *ct* (fig 2, cluster 1), some to *ll* (fig 2, cluster 2), and to some *gr* (fig 2, cluster 3).

The differences between our and Vareschi's results are probably due to differences in techniques: sorting out subunits from direct stimulus response *versus* adapting out responses using conditioning and test stimuli. The difficulty with our technique is that we could not be sure that our subunits correspond to the responses of single neurons, a question that we address further below. On the other hand, Vareschi's technique also has the problem that several cells may be responding to his stimuli, not all of which – as discussed above – are adapted out by the same stimuli. Further, Vareschi recorded primarily from drone antennae while we recorded exclusively from worker antennae. Finally, many of the details of Vareschi's analysis are not reported with his results, so it is difficult to fully evaluate his data.

RESPONSE TO BINARY ODORS

Most odors emanating from biological organisms such as plants, flowers or animals are complex blends of odorants. Even sex and aggregation pheromones in insects are often blends of 2 or more odorants (Wood *et al,* 1967; Klun *et al,* 1973; Linn *et al,* 1984). The way an organism perceives the odor quality of blends may be very different from the way it perceives the quality of the component odorants (Derby and Ache, 1984; Getz and Smith, 1987; but see Rabin and Cain, 1989). This difference could be due to nonlinear phenomena at

Table I. Correlation matrix of tuning characteristics of whole placode and subplacode response units with respect to the odorants undecanoic acid *(ud)*, dodecanoic acid *(dd)*, linalool *(ll)*, limonene *(li)*, geraniol *(gr)*, and citral *(ct)* at a concentration level of 10 μg/μl.

Level	*Odorant*	ud	dd	ll	li	gr
Placode [a]						
	dd	0.42				
	ll	0.18	0.29			
	li	0.51	0.63	0.22		
	gr	0.07	0.32	–0.26	0.17	
	ct	0.24	0.11	–0.20	0.15	–0.25
Subplacode [b]						
	dd	0.34				
	ll	0.27	0.36			
	li	0.40	0.33	0.27		
	gr	0.34	0.30	0.08	0.38	
	ct	0.30	0.39	0.27	0.27	0.23

[a] Data obtained from responses of 70 placodes; [b] data obtained from sorting 15 of the 70 placodes into 55 subplacode units, where the 15 were selected at random.

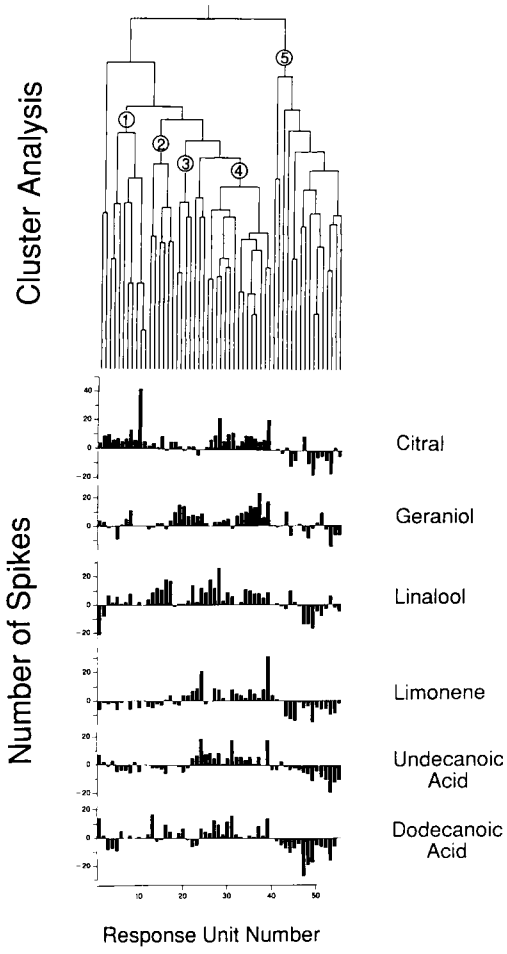

Fig 2. A cluster analysis of the normalized net response of 55 subplacode units to the 6 aromatic and fatty acid odorants listed in table I. Circled numbers of this dendrogram refer to clusters discussed in Akers and Getz (1992). (Reproduced by permission of the latter).

the peripheral level, such as synergistic or inhibitory responses of sensory neurons to blends when compared with responses to the individual odorants in the blend.

We investigated the existence of such nonlinearities by focusing on the simplest level of interaction: that of 50%:50% blends of 2 odorants (Akers and Getz, 1993). Specifically, as baseline data, we obtained the response of placodes to selected odorants O_1, O_2..., at given concentrations and then to binary mixtures with each component at half the given concentration. In this way, if stimulus 1 was odorant O_1 at concentration c – *ie*, $\omega_1 = [O_1]_c$, stimulus 2 was odorant O_2 at concentration c – *ie*, $\omega_2 = [O_2]_c$, and stimulus 3 was a 50%:50% blend of these 2 odorants – *ie* $\omega_3 = \omega_1/2 + \omega_2/2$ (where $\omega_i/2 = [O_i]_{c/2}$, i = 1,2) and if O_1 and O_2 were to be the same odorant, then $\omega_1 = \omega_2 = \omega_3$. On the other hand, if the response of a sensory unit to ω_1 was greater than its response to ω_2 and, then – assuming the response increases more for each additional small unit concentration of O_1 than for each additional small unit concentration of O_2 added (in the log-linear range above threshold and below saturation) to either ω_1 or ω_1 – we expected the response to ω_3 to lie in between the responses to ω_1 and ω_2. Exactly where the response to ω_3 should lie in relation to the responses to ω_1 and ω_2 is a complicated question that requires some knowledge about the transduction systems of the response units (in the context of taste see McBride, 1989). For a single sensory neuron, however, when the response to ω_3 exceeded the response to ω_1 and ω_2 then, the above asumption was not satisfied and we concluded that we had some type of synergistic interaction. Similarly, when the response to ω_3 fell short of the responses to ω_1 and ω_2 then the above assumption was not satisfied and we concluded that we had some type of inhibitory interaction (Akers and Getz, 1993).

We conducted studies of response to mixtures using 2 sets of 4 odorants, each at 2 different concentrations, a 2 μg/μl concentration (which we regarded as moderately weak) and a 60 μg/μl concentration (which we regarded as moderately strong)

(Akers and Getz, 1993). Each placode was exposed to these 4 odorants, their 6 50%:50% binary blends, and their 1 25%:25%25%:25% quaternary blend. The 2 sets of odorants were: i) an aromatic group comprising linalool *(ll)*, limonene *(lm)*, geraniol *(gr)*, and citral *(ct)*, which are components of floral odors (von Frisch, 1967) and honey bee aggregation pheromones (Free, 1987); ii) an octyl group comprising 1-octanol (1-ol), 2-octanol *(2-ol)*, octanal *(al)*, and 2-octanone *(2-one)*, which are components of plant and floral odors. 1-Octanol is also known to be part of the alarm pheromone (Free, 1987).

Placodes were either stimulated with the aromatic odors or octyl odors but not both. Many placodes received stimuli at both concentrations (≈ 27 stimuli: 11 at each of 2 concentrations and a control every 5–6 stimuli to check that the responsiveness of the placode had not changed over time). For the aromatic set, 26 placodes received stimuli at the 2 μg/μl level, 28 at the 60 μg/μl level, and 17 of these at both concentrations. For the octyl set, 31 placodes received stimuli at the 2 μg/μl level, 34 at the 60 μg/μl level, and 23 of these at both concentrations.

Placode response was sorted, as described above, into 3 or 4 subplacode response units. Correlation analyses were undertaken of subplacode response levels to the 4 odorants in each stimulus group (tables II and III). Again the aromatic data (table II) did not support Vareschi's tuning classification scheme. The aromatic data do indicate that some patterns at the subplacode level are consistent across the 3 concentrations in question (*ie*, 10 μg/μl in table I and 2 μg/μl and 60 μg/l in table II). For example, scanning the linalool column for the subplacode results, we see that responses to linalool correlate somewhat with response to limonene and citral, but not to geraniol. The octyl data (table III), however, do indicate that the responses to the 2 alcohols, 1-octanol and 2-octanol, are somewhat correlated at the subplacode level relative to most of the other comparisons, as are the responses to octanal (≡ '1-octanone') and 2-octanone; but

Table II. Correlation matrix of tuning characteristics of whole placode and subplacode response units with respect to the odorants linalool *(ll)*, limonene *(li)*, geraniol *(gr)*, and citral *(ct)* at a concentration level of 2 and 60 μg/μl.

Level	*Odorant*	ll	lo *(2 μg/μl)*	gr	ll	li *(60 μg/μl)*	gr
Placode [a]							
	li	0.18			0.19		
	gr	−0.08	0.21		−0.03	0.13	
	ct	−0.08	0.34	−0.03	−0.28	0.00	0.19
Subplacode [b]							
	li	0.20			0.21		
	gr	−0.10	0.24		0.00	0.28	
	ct	0.09	0.26	0.12	0.22	0.12	0.13

[a] Data obtained from responses of 26 and 28 placodes respectively at the 2 and 60 μg/μl concentrations; [b] data obtained from responses of 78 and 84 subplacode units respectively sorted from the 26 and 28 placodes mentioned in footnote [a].

Table III. Correlation matrix of tuning characteristics of whole placode and subplacode response units with respect to the odorants 1-octanol *(1-ol)*, 2-octanol *(2-ol)*, octanal *(al)*, and 2-octanone *(2-one)* at a concentration level of 2 and 60 μg/μl.

Level	*Odorant*	1-ol	2-ol *(2 μg/μl)*	al	1-ol	2-ol *(60 μg/μl)*	al
Placode [a]							
	2-ol	−0.26			0.26		
	al	−0.40	−0.57		−0.62	−0.76	
	2-one	−0.42	−0.17	0.34	−0.39	−0.40	0.29
Subplacode [b]							
	2-ol	0.26			0.15		
	al	−0.03	−0.08		0.06	−0.01	
	2-one	0.01	0.26	0.18	0.11	0.03	0.15

[a] Data obtained from responses of 26 and 28 placodes respectively at the 2 and 60 μg/μl concentrations; [b] data obtained from responses of 78 and 84 subplacode units respectively sorted from the 26 and 28 placodes mentioned in footnote [a].

the correlations between responses to octanal and the alcohols are either very small or negative.

The 4 11 x 11 correlation matrices for the 4 sets of odorants and their binary and quaternary combinations were also generated. Surprisingly, correlations between binary odors and their 2 odorant components were not very large and were not much larger than the correlations between binary odors and the 2 remaining odorants in the group that were not the components of the odorants in question (Akers and Getz, 1993). Specifically, the average component and non-component correlations for the 4 groups of odorants were respectively (see Akers and Getz (1993), for individual values): 2 μg/μl aromatics: 0.16 and 0.14; 60 μg/μl aromatics: 0.22 and 0.11; 2 μg/μl octyl: 0.20 and 0.13; 60 μg/μl octyl: 0.22 and 0.11.

Considering these values, it is not surprising that our principal components analysis of the response of the subplacodes to the 11 stimuli did not indicate that the data could be represented reasonably well in a 4-dimensional space (Akers and Getz, 1993) in which the principal factors were the odorants themselves. In fact the only obvious grouping that emerged from the principal components analysis was that the greatest amount of variation was explained by ordering placodes along an axis that contrasted excitatory and inhibitory response to the stimuli (Akers and Getz, 1993).

To investigate the existence of obvious inhibitory or synergistic interactions, we used a cluster analysis to identify groups of subplacode response units (4–8 units per group) that were similarly tuned (Akers and Getz, 1993). The data were aggregated within groups to obtain mean and standard error response levels, and then obvious inhibited or synergized responses to binary combinations were identified (figs 3–6). More synergistic responses to binary combinations were identified than would be expected at random. If a placode subunit, however, really represented the activi-

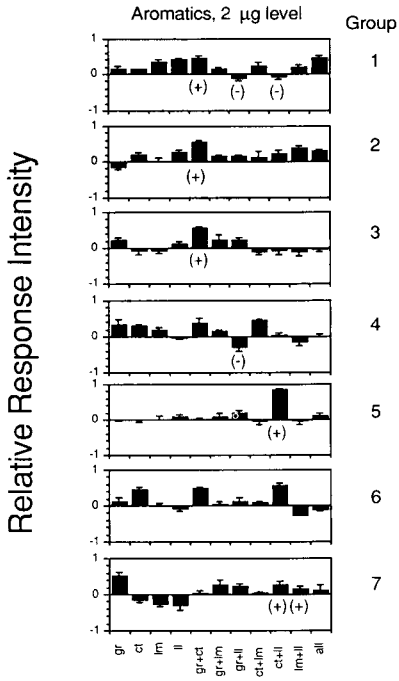

Fig 3. The mean (± SEM) normalized net responses of groups of placodes to the 11 2 µg/µl aromatic stimuli listed in table II. For the binary odors, the (+) and (–) respectively designate significant ($p < 0.05$) synergistic and inhibitory interactions according to definitions presented in the text.

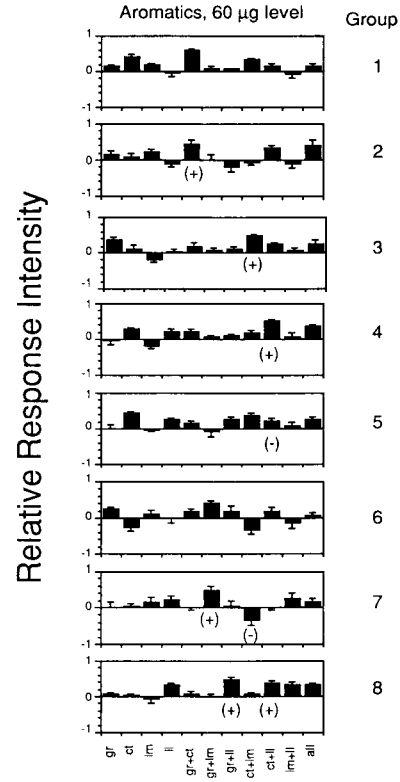

Fig 4. The mean (± SEM) normalized net responses of groups of placodes to the 11 60 µg/µl aromatic stimuli listed in table II. For the binary odors, the (+) and (–) respectively designate significant ($p < 0.05$) synergistic and inhibitory interactions according to definitions presented in the text.

ty of 2 olfactory sensory neurons, one of which responded primarily to O_1 and the other primarily to O_2 then nonsynergistic responses of 2 neurons added together could be misinterpreted as a synergistic response of a single neuron. This follows because of log-linear-like relationships between the response of olfactory sensory neurons and odorant concentrations within threshold and saturation levels (as reviewed by Kaissling (1987), Mayer *et al* (1987), and by Masson and Mustaparta (1990); see also Boeckh and Ernst (1987) and Fujimura *et al* (1991)), the number of spikes fired per unit interval by an olfactory sensory neuron in response to $[O_1]_c$ would be considerably more than half the number of spikes fired in response to $[O_1]_{c/2}$. The same would true in the context of O_2. Thus if a subplacode consisted of 2 sensory neurons, one sensitive to O_1 and the other to O_2, then we would expect to observe the response to $[O_1]_{c/2} + [O_2]_{c/2}$ to be greater than the response of the subplacode unit

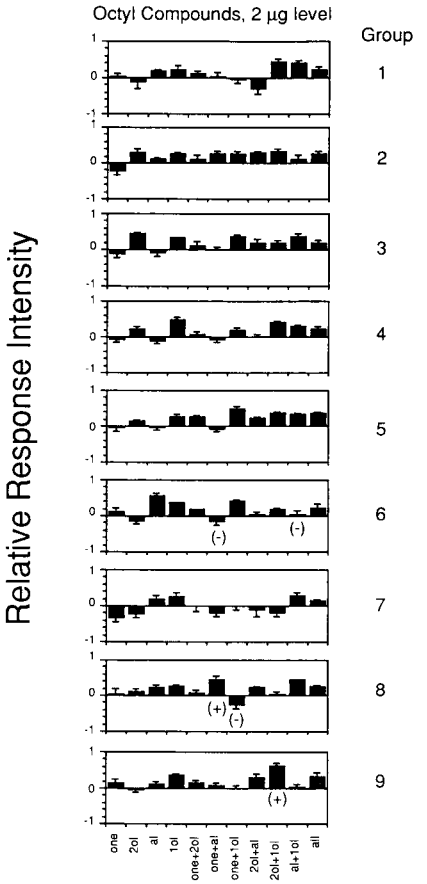

Fig 5. The mean (± SEM) normalized net responses of groups of placodes to the 11 2 µg/µl octyl stimuli listed in table III. For the binary odors, the (+) and (–) respectively designate significant ($p < 0.05$) synergistic and inhibitory interactions according to definitions presented in the text.

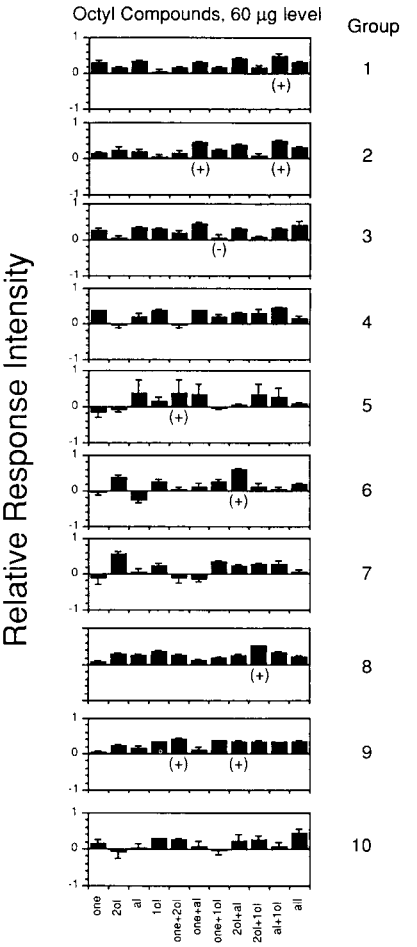

Fig 6. The mean (± SEM) normalized net responses of groups of placodes to the 11 60 µg/µl octyl stimuli listed in table III. For the binary odors, the (+) and (–) respectively designate significant ($p < 0.05$) synergistic and inhibitory interactions according to definitions presented in the text.

to $[O_1]_C$ or $[O_2]_C$. Thus some of the synergisms might be explained by subplacode groups consisting of 2 or more specialized sensory neurons (if each neuron responded reasonably well to both compounds, then this argument would not work). The fact that not many synergisms are evident in the data set, however, suggests that the broad tuning of the subplacode responses, even if the

subplacode represents the response of more than 1 sensory neuron, is due to the broad tuning of individual sensory neurons rather the combined response of 2 or more different types of specialized sensory neurons.

A few obvious inhibitory interactions were also identified, although in ≈ 200 comparisons one would expect some significant results to arise purely by chance. Two of these inhibitory interactions indicated that responses at or above control responses to both odorants tested separately, could be inhibited to below control responses in the case of the corresponding binary combination (*eg* group 7, fig 4 and group 6, fig 5). Inhibitory interactions have been identified in the sensory neurons of other organisms with sensilla containing only 2 cells (Derby and Ache, 1984). In the more complex placode sensilla of honey bees, however, both inhibitory and synergistic interactions could be taking place simultaneously, and cancelling one another out at the subplacode level.

In general, the question of synergistic or inhibitory responses of units to blends compared with their responses to individual component odorants is very difficult to address without more details on whether odorants are competing for the same or different membrane receptor sites and, if the latter holds, whether these receptor sites are on the same or different sensory neurons. The matter is further complicated by the fact that the sensory neurons in the same placode may be influencing each other's firing behavior, as discussed below, and perhaps even inhibiting one another's response (White *et al,* 1990).

ORGANIZATION OF PLACODES

A null hypothesis model for the organization of the subplacode response units identified in the previous sections is that the subplacode units are distributed randomly among the placodes subject to the constraint that each placode has 3 or 4 subplacode units. This hypothesis is easily tested by considering the differences in tuning, as determined by equation [3], between any 2 subplacode units j and k picked at random, subject to the constraint that they come from the same placode ($\cos\theta_{jk}$ within placode), compared with the constraint that they come from different placodes ($\cos\theta_{jk}$ between placode).

For each of the 5 data sets (table IV), we generated mean values for within and between placode measures of tuning similarity by systematically going through the placodes and selecting one of its subplacode units at random. This subplacode unit was then paired with the remaining 2–3 subplacode units in the same placode, as well as an equal number of subplacode units selected at random from the other placodes. Using equation [3], within and between placode inner products were generated for these matched pairs, generating a sample of within and between values from which the means could then be calculated. The results in table IV indicate that similarly tuned subplacode units are more likely to be placed in separate placodes than would be expected from a random placement of subplacode units (none of the individual row entries are significant at the $p < 0.05$ entry, but a simple sign test indicates that the overall results is significant at this level – see also Akers and Getz (1993), where individual row entries are shown to be significant for the full data set of 11 stimuli – odorant + binary + quaternary – rather than the 4 pure odorant stimuli considered here).

Insight into how subplacode units might be distributed among placodes as a function of their tuning can be obtained by comparing correlations in the response of placodes (whole) and subplacode units to

Table IV. Within and between placode comparisons of mean tuning differences between matched pairs of subplacodes.

Data set	N [a]	$\overline{cos\theta}$ *Within* [b]	$\overline{cos\theta}$ *Between* [b]
10 μg/μl aromatic and fatty acid [c]	40	0.00 (± 0.09)	0.16 (± 0.07)
2 μg/μl aromatic [d]	53	−0.02 (± 0.08)	0.19 (± 0.06)
60 μg/μl aromatic [d]	52	−0.01 (± 0.07)	0.11 (± 0.08)
2 μg/μl octyl [e]	63	0.18 (± 0.07)	0.25 (± 0.06)
60 μg/μl octyl [e]	70	0.36 (± 0.05)	0.45 (± 0.06)

[a] Sample size; [b] mean (± SE) correct to 2 decimal places over sample of $cos\theta_{jk}$ calculated according to [3]; [c] see table I or figure 2 for list of 6 stimuli associated with this data set; [d] see table II for list of 4 stimuli associated with this data set. This data differs from that in table I in Getz and Akers (1993) since the latter is based on tuning with respect to the full set of 11 stimuli comprising 4 odorant, 6 binary blends and 1 quaternary blend; [e] see table III for a list of 4 stimuli associated with this data set. This data differs from that in table I in Getz and Akers (1993) for the reasons given in [d].

the various sets of odorants. To begin with, tables I, II and III indicate that while correlations at the subplacode level are generally positive, ranging between −0.10 and 0.40, correlations at the whole placode level are wider, ranging – from −0.76 to 0.51 – and some unexpectedly large negative correlations (*ie* < −0.25) are evident. (Note that the correlations presented in tables I and II are not identical to the same correlations presented in Akers and Getz (1993): in computing tables II and III, the raw data are normalized with respect to response to the 4 odorants in question before the correlations are calculated, while in Akers and Getz (1993) the same raw data are first normalized with respect to the 11 single odorant, binary, and quaternary blends before the correlations are calculated.)

To help interpret these correlation results, consider the 2-dimensional example illustrated in figure 7. In this example, the correlation between the responses of subplacode unit 1 and 2 to odorants A and B is positive in both cases. However – be-

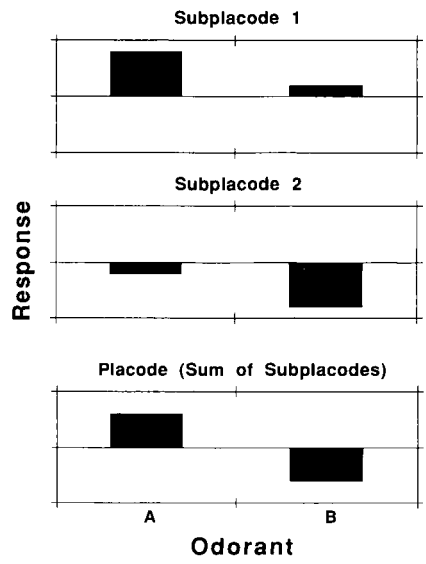

Fig 7. Illustrative data indicating how positive correlations in the responses of subplacode units 1 and 2 to odors A and B result in a negative correlation of the whole placode to odors A and B (see text for discussion).

cause subplacode unit 1 is excited by both odorants but more strongly by A than B, while subplacode unit 2 is inhibited by both odorants but more strongly by B than A – the whole placode exhibits a positive response to A and a negative response to B. Thus the correlation between the response of the whole placode to both odorants A and B is negative. Since we already know, as discussed in the previous section, that the factor explaining most of the variation is one that categorizes placodes into those that are primarily excited by all the odorants and those that are primarily inhibited by all the odorants (Akers and Getz, 1993), the correlation coefficients suggest that subplacode units might be placed in placodes so that each placode is much more likely to have a representative sample of primarily excitatory and primarily inhibitory subplacode units than would be expected at random. This is consistent with the result in table IV that subplacode units are more dissimilar within placodes than across placodes.

The fact that placodes are structured in a way that contrasts the tuning of subplacode units, rather than in a way that clumps similarly tuned subplacode units into the same placode suggests that the placodes themselves could be the first level at which odorant quality computations take place. If coordinated firing of subplacodes within the same placode can be demonstrated, then we have evidence that the placodes themselves function at a level beyond that of pure input transduction devices. The hypothesis that subplacode units are firing independently can be tested by comparing the statistics of interspike interval variables at the subplacode and whole placode levels, as discussed in the *Data analysis* section.

The mean interspike interval (MII) data in table V has 2 particularly interesting results. First, the MII coefficients of variation (CVs) are consistently higher at the subplacode level than the placode level – that is, the mean spike intervals are more uniform for the whole placode than for the subplacode units. Thus, it appears that responses of subplacode units are coordinated within placodes. Second, among the subplacode units the value of the CVs appears to be independent of the size of the MII, while among placodes a linear relationship between CVs and MIIs is apparent (see footnotes [a] and [b] to table V; and also Getz and Akers, unpublished data). These 2 results give us confidence that our method for sorting placode responses into subplacode response units is a meaningful procedure, because random subsamples of placode data would have the same statistical characteristics as the whole data set.

Another way of looking at the organization of the placodes is to assess whether some placodes contain more specialized units than others and, if so, whether clumping of specialized *versus* generalized subplacode units is related in any way to the degree of specialization of the placode itself or to the average tuning distance between subplacode units within placodes. Specifically, if the response of the j th placode has been sorted into 4 subplacode response units, j_1, j_2, j_3 and j_4, and of the 4 j_2 is selected at random then, using equation [3], we define:

$$\overline{\cos\theta_j} = \frac{\cos\theta_{j_2 j_1} + \cos\theta_{j_2 j_3} + \cos\theta_{j_2 j_3}}{3} \quad [6]$$

and, using equation [4], we define

$$\overline{H_j} = \frac{\Sigma_{i=1}^{4} H_{ji}}{4} \quad [7]$$

Note that the quantity H_j is different from $\overline{H_j}$: (i) H_j represents the degree of speciali-

Table V. Mean-interspike-intervals (MII) measured in ms and corresponding percent coefficients of variation (CV) in parentheses obtained from spike train representation of responses of placodes and subplacode units to various stimuli.

Odor stimulus	*Subplacode* [a] (2 μg/μl)	*Placode* [b] (2 μg/μl)	*Subplacode* [a] (10 μg/μl)	*Placode* [b] (10 μg/μl)	*Subplacode* [a] (60 μg/μl)	*Placode* [b] (60 μg/μl)
Octyl set						
Mineral oil [c]	36.4 (90.1)	12.4 (76.3)			36.4 (90.1)	12.4 (76.3)
2-Octanone	32.6 (87.8)	11.3 (71.2)			25.7 (85.7)	7.4 (76.7)
2-Octanol	35.2 (85.9)	9.4 (71.4)			21.7 (91.1)	6.8 (67.4)
Octanal	35.8 (86.8)	11.5 (75.6)			22.1 (88.9)	6.9 (70.0)
1-Octanol	27.8 (92.3)	8.9 (72.3)			21.7 (90.3)	6.8 (63.9)
Aromatic and fatty acid set						
Mineral oil [c]	33.5 (95.3)	12.6 (78.1)	31.6 (82.3)	11.4 (74.1)	33.5 (95.3)	12.6 (87.1)
Geraniol	33.6 (88.3)	12.2 (72.9)	38.6 (88.4)	8.8 (62.8)	30.8 (90.1)	9.5 (72.9)
Citral	35.0 (85.7)	10.7 (73.2)	26.8 (81.5)	8.8 (63.8)	25.4 (81.9)	8.2 (68.6)
Limonene	34.4 (85.0)	12.1 (74.9)	25.8 (86.3)	11.7 (75.0)	31.4 (85.0)	10.2 (74.2)
Linalool	37.5 (82.3)	11.2 (73.3)	28.4 (85.8)	8.4 (59.6)	29.1 (88.2)	9.0 (71.1)
Undecanoic acid			32.0 (80.6)	11.3 (68.9)		
Dodecanoic acid			33.8 (86.4)	11.9 (75.1)		

[a] Data obtained from sample of 56–105 subplacode response profiles, depending on stimulus. The regression of CV on MII is CV = 89.4(4.6) – 0.08(0.15) x MII. The SE are in parentheses and the slope is not significantly different from 0; [b] data obtained from sample of 15–35 whole placode response profiles, depending on the stimulus. The regression of CV on MII is CV = 56.1(4.2) + 1.53(0.42) x MII. The SE are in parentheses and the slope is significantly different from 0 ($p < 0.01$); [c] this mineral oil control (fig 1) is the same for the 2 and 60 μg/μl concentrations, because the same placodes were stimulated with odorants at both concentrations.

zation of the jth placode, as defined by equation [4], before the response of the jth placode is sorted into subplacode response units; (ii) H_j is calculated after the data has been sorted into subplacode response units and is the mean H_{ji} across subplacodes $i = 1,\ldots,k$ (in our case $k = 3$ or 4) within the same placode.

No relationship is evident between $\cos\theta_j$ and H_j over the sample of placodes $j = 1,2\ldots$, and the sets of odorants we studied (table VI), except for the set which contained the fatty acids. The values of $\cos\theta_j$ and H_j, however, are dependent on the particular odorants used, so that relationships between these 2 measures may exist for some sets of stimuli and not others.

ODOR QUALITY COMPUTATIONS

One of the central issues of olfactory research is elucidating how olfactory systems compute the quality of blends of odorants. The following is a skeletal description of the essential features of an insect olfactory system capable of identifying nonpheromonal olfactory stimuli (Getz, 1991; Getz and Page, 1991):

Table VI. Relationship between the average tuning differences between and the average degree of specialization of the subplacode units in the same placode.

Data set	N [a]	*Average* $\overline{\cos\theta_j}$ *(Expression [6])*	*Average* $\overline{H_j}$ *(Expression [7])*	*Regression relationship* [b] *Slope*	*Significance*
10 μg/μl aromatic and fatty acid [c]	14	−0.03 (± 0.06)	0.82 (± 0.02)	− 0.26 (± 0.07)	$p < 0.01$
2 μg/μl aromatic [d]	26	−0.05 (± 0.07)	0.80 (± 0.02)	0.04 (± 0.06)	NS
60 μg/μl aromatic [d]	28	0.05 (± 0.06)	0.83 (± 0.02)	0.08 (± 0.06)	NS
2 μg/μl octyl [e]	31	0.16 (± 0.07)	0.83 (± 0.01)	0.05 (± 0.03)	NS
60 μg/μl octyl [e]	34	0.32 (± 0.05)	0.88 (± 0.01)	0.02 (± 0.03)	NS

[a] Number of placodes in sample; [b] the regression relationship is of the form $\overline{H_j} = a\,\overline{\cos\theta_j} + b$. The slope a is listed with its standard error in parentheses. These values and the significance were obtained using the REG procedure in SAS; [c] see table I or figure 2 for list of 6 stimuli associated with this data set; [d] see table II for list of 4 stimuli associated with this data set; [e] see table III for list of 4 stimuli associated with this data set.

– individual olfactory sensory neurons respond at a level influenced by 2 factors: i) the average intensity (concentration); and ii) the average quality (ratio of component odorants) of a stimulus impinging over a short interval of time (sampling or sniff cycle; see Getz, 1991) upon the antennae of an individual. If the number of odorants that at least one of the sensory neurons responds to is μ, then the ith stimulus has a representation $\omega_i = (\omega_{i\,1},\ldots,\omega_{i\mu})' \in R^{\mu+}$ (the positive quadrant of the Euclidean vector space R^{μ}).

– Tens of thousands of sensory neurons converge on hundreds of glomeruli where their activity is summed within glomeruli to reduce noise due to sampling and stochastic neuronal activity. Inhibitory neurons are also known to exist within and between glomeruli (Flanagan and Mercer, 1989). This may serve to contrast the activity of the glomeruli and hence the firing pattern in the relay neurons that ascend, typically one from each glomerulus (*ie*, excluding the macroglomeruli in drones), inside the antennoglobular tract (AGT) to higher centers of the brain (*eg* mushroom bodies in the protocerebrum).

– The firing rates of these v relay neurons, as a consequence of stimulus ω_i applied over a single sniff cycle, can be represented by a vector in $\rho_i = (\rho_{i\,1},\ldots,\rho_{iv})'$ in a v-dimensional olfactory sensory space. The direction of this vector ρ_i then represents the 'computed' quality of the odor stimulus which, once generated, may be associated with stimuli from other modalities, or identified as similar to a stored memory pattern, or used to trigger motor output because of innate or learned behavior (Getz, 1991). The transformation function $\mathcal{T}(\cdot)$ representing the mathematical operation

$$\rho_i = \mathcal{T}(\omega_i), \qquad [8]$$

is a model of how the peripheral and deutocerebral olfactory systems process olfactory information.

– The integrity of the olfactory perceptual system requires that the 'perceived' quality of a stimulus must be invariant over at least a small range of stimulus concentra-

tions. The simplest model possessing this quality is to assume that transformation function $\tau(\bullet)$ is a matrix operation T, reducing equation [8] to the linear equation $\rho_i = T\omega_i$ or, more appropriately, to the log-linear equation

$$\rho_i = T \ln\omega_i, \qquad [9]$$

since sensory neurons are known to have an approximately linear response to the logarithm of the concentration of odorants (Boeckh and Ernst, 1987; Kaissling, 1987; Mayer *et al,* 1987; Masson and Mustaparta, 1990; Fujimura *et al,* 1991). (Note that we use the notation $\ln\rho_i = (\ln\rho_{i1},\ldots, \ln\rho_{i\mu})'$). This log-linear model is inadequate for at least 2 reasons: i) threshold and saturation effects limit the range of concentrations over which a log-linear representation is valid; and ii) it does not include synergistic or inhibitory phenomena.

Suppose, however, that equation [9] was an adequate model over a range of logarithmically transformed stimulus concentration. The question remains how to construct the matrix T. The column entries of T actually represent the output from each glomerulus (*ie* the firing rate of its ascending relay neuron) to a standardized dosage of the odorants that span the stimulus input space $R^{\mu+}$ (Getz and Chapman, 1987). If each glomerulus represented the summed input of a class of similarly tuned neurons, such as those depicted in figures 3–6, then the matrix T could be constructed by sorting receptors into tuning class categories. No data, however, is available to support the hypothesis that all similarly tuned olfactory sensory receptors arborize in the same glomerulus.

Another difficulty is that we do not know the appropriate procedure for determining the tuning category of a particular receptor. In the data presented above, we implicitly assumed that odorant quality information is contained in the difference between the response of the receptor to the odorless control and the odor-laden stimuli. Each sensory neuron, however, is part of a neural network in which synapses between neurons are usually characterized by a threshold: if the presynaptic neuron is firing below threshold, then the postsynaptic neuron does not respond. In figure 8 we see how the tuning direction of a neuron is determined by the threshold. Further, even

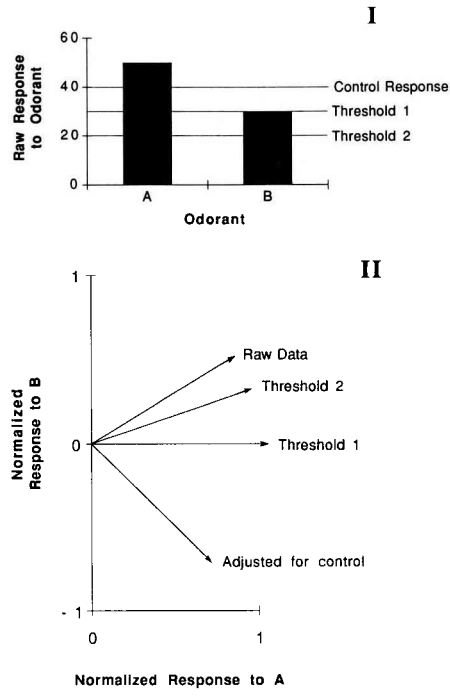

Fig 8. The net response of a sensory neuron depends on the value that we subtract from the actual or raw response. In the above illustrative case, the response to odorants A and B, indicated in table I, are respectively above and below the response to the odorless mineral oil control. This case implies that the normalized tuning vector illustrated in table II represents a point in the 4th quadrant. If the real contribution of this neuron to a glomerular network was determined by threshold 1 or 2, then the tuning direction would be given by the corresponding vectors illustrated in table II.

if we knew the value of the threshold, neural networks are highly nonlinear so that the contribution of single neurons cannot be evaluated in isolation from the total network response. Thus, although an evaluation of the tuning spectra of sensory neurons provides valuable insight into the structure of the peripheral component of an olfactory system, the contribution of these sensory neurons to the overall filtering characteristics of the neural processes afferent to the AGT cannot be evaluated without knowing how they wired into the olfactory neural network.

FUTURE DIRECTIONS

From the above discussion, it is obvious that 2 outstanding issues remain to be resolved in our understanding of how the response characteristics of sensory neurons in the honey bee placode might contribute to the perception by individuals of the quality of olfactory stimuli. First, we need to record from single sensory neurons and reconcile the data so obtained with the data that we have on subplacode response units: are they in fact the same or do our subplacode response units generally correspond to output from more than one cell? The lack of synergisms in the response of subplacodes to binary odors (figs 3–6), and our interspike interval data suggest that subplacode units may in fact be single cells. Second, we need to unravel the anatomy of sensory cells and understand how they arborize in the deutocerebrum as a function of their tuning characteristics. This study may help us resolve how best to choose a threshold value for the purposes of evaluating the tuning characteristics of the sensory neurons.

Once we have an idea of how the olfactory sensory neurons arborize within the glomeruli, we can then begin to build a model of the olfactory system from the bottom up based on the structure of interneurons among and within these glomeruli. In order to evaluate the performance of such a model, we also need to measure the response of the ascending relay neurons to a spectrum of stimuli presented over a range of concentrations. It would be highly desirable to have simultaneous measurements from several ascending neurons, but methods for recording such data need to be developed. Of special importance will be to characterize the delays involved in the onset of stimulation and the response of the relay neurons, as well as the phasic properties of the response. These data will help us infer how many layers of neurons or feedback loops might be involved in the computation by the deutocerebrum of quality of an odor, although the actual quality of stimulus may only be interpreted contextually in parts of the protocerebrum, such as the mushroom bodies (Menzel, 1990).

ACKNOWLEDGMENTS

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Résumé — Caractéristiques de la réponse olfactive et de la sélectivité des placodes chez l'abeille, *Apis mellifera* L. On sait peu de choses concernant la détection des odeurs non phéromonales par les neurones sensoriels de l'abeille parce que :

– isoler l'activité individuelle des neurones récepteurs olfactifs est techniquement difficile et que

– la majorité des études portant sur l'olfaction des insectes s'est focalisée sur les questions relatives à la détection des phéromones sexuelles ou d'agrégation plutôt

qu'à celle des odeurs ordinaires. Nous discutons ici de la détection des odeurs ordinaires dans un contexte général. Nous présentons les méthodes pour définir les caractéristiques de la sélectivité d'un neurone sensoriel vis-à-vis d'un ensemble de stimuli odorants, en utilisant une analyse quantitative des potentiels d'action dérivée de la réponse d'une placode *(sensilla placodea)* entière ou d'une sous-unité définie de placode (celle-ci représentant probablement la réponse individuelle d'un neurone sensoriel) à ces stimuli odorants. Nous présentons ensuite les méthodes pour évaluer la différence de sélectivité entre 2 sous-unités de placodes et le degré de spécialisation des sous-unités de placodes.

Nous passons également en revue les analyses de données récemment publiées (Akers et Getz, 1992), et nous utilisons certaines de ces données pour avoir de nouveaux éléments sur l'organisation des placodes par rapport aux caractéristiques de sélectivité des sous-unités de placodes. Nous discutons d'abord les catégories de cellules réceptrices proposées par Vareschi (1971) et montrons que ces catégories ne coïncident pas avec nos données. Nos données montrent une variabilité énorme dans les caractéristiques de sélectivité des sous-unités de placodes et une absence de catégories bien définies. Nous passons aussi en revue la question de la relation entre la réponse des sous-unités de placodes aux composés odorants pris individuellement et la réponse de ces neurones à des combinaisons binaires de ces produits odorants, en particulier dans le contexte de phénomènes d'inhibition et de synergie. Les modèles appropriés pour analyser ces phénomènes sont extrêmement complexes, mais nos données montrent que ces phénomènes semblent exister, bien qu'à un degré limité.

Notre analyse de la répartition des sous-unités de placodes au sein de placodes, en termes de caractéristiques de sélectivité et de degré de spécialisation des sous-unités de placodes, montre que celles-ci ne sont pas distribuées au hasard au sein des placodes mais sont placées de sorte à accroître le contraste entre les sous-unités de placodes au sein de chaque placode. De plus, nous utilisons le coefficient de variation des intervalles de potentiel d'action pour prouver que les sous-unités de placodes dans la même placode ne déchargent pas indépendamment les unes des autres.

Nous concluons avec une discussion générale sur l'identification de la qualité des odeurs non phéromonales et sur les futurs axes de recherche nécessaires pour résoudre certaines des questions encore en suspens.

neurone sensoriel / codage olfactif / électrophysiologie / *sensilla placodea* / olfaction

Zusammenfassung — Die olfaktorische Codierung und Abstimmung der chemo-rezeptiven Strukturen in den antennalen Plakoden-Sinnesorganen der Honigbiene *Apis mellifera*. Nur wenig ist bisher über die Erkennung «gewöhnlicher» (also nicht-pheronaler) Gerüche durch sensorische Neurone der Honigbiene bekannt, einmal weil es technisch schwierig ist, die Aktivität einzelner olfaktorischer Rezeptor-Neurone zu messen, zum anderen, weil sich die meisten Untersuchungen über den Geruchssinn der Insekten auf Geschlechts und soziale Gerüche konzentrieren und nicht auf «gewöhnliche» Gerüche. Hier diskutieren wir das Problem der Erkennung «gewöhnlicher» Gerüche in einem allgemeinen Zusammenhang. Wir stellen Methoden vor, welche die Abstimmung sensorischer Neurone in Hinblick auf ein Bündel von Geruchsreizen zeigt, indem wir Daten von Spikes-Zählungen als Antwort

einer ganzen Plakode und einer Subplakode auf einen bestimmten Geruch auswerten. Die Subplakode repräsentiert wahrscheinlich die Antwort eines einzelnen Sinnesneurons. Anschließend beschreiben wir Methoden zur Bewertung des Unterschiedes in der Abstimmung zwischen zwei Einheiten von Subplakoden und dem Grad der Spezialisierung von Subplakoden-Einheiten.

In dieser Arbeit geben wir außerdem eine Übersicht über kürzlich publizierte Daten (Akers und Getz, 1992) und wir verwenden einige dieser Daten zur Analyse der Organisation der Plakoden-Sinnesorgane in Hinblick auf die olfaktorische Abstimmung der Subplakoden-Einheiten. In dieser Übersicht diskutieren wir zunächst die von Vareschi (1974) vorgeschlagene Einteilung der olfaktorischen Abstimmung in sensorischen Neuronen, und zeigen, daß sich seine Kategorien mit unseren Daten nicht vereinbaren lassen. Unsere Daten zeigen eine beträchtliche Variabilität in den olfaktorischen Abstimmungseigenschaften der Subplakoden-Einheiten, aber keine klar abgegrenzten Kategorien. Wir geben auch eine Übersicht über die Frage der Beziehungen zwischen der Antwort der Subplakoden-Einheiten auf Einzelgerüche und die Antwort dieser Neurone auf binäre Kombinationen dieser Gerüche, besonders in Hinblick auf inhibitorische und synergistische Phänomene. Geeignete Modelle zur Analyse dieser Phänomene sind extrem kompliziert, aber unsere Daten zeigen, daß diese Phänomene existieren, allerdings nur in einem begrenzten Ausmaß.

Die Analyse der Verteilung der Subplakoden-Einheiten innerhalb der Plakoden zeigen in Hinblick auf die olfaktorische Abstimmung und auf den Grad der Spezialisierung dieser Einheiten, daß die Subplakoden-Einheiten nicht zufällig in den Plakoden verteilt sind, vielmehr scheinen Subplakoden-Einheiten innerhalb jeder Plakode unterschiedlicher zu sein als Subplakoden verschiedener Plakoden-Sinnesorgane. Weiter benutzen wir den Variations koeffizienten der Daten über die Interspike-Intervalle für den Nachweis, daß die Subplakoden-Einheiten in derselben Plakode nicht unabhängig voneinander erregt sind.

Zum Abschluß der Arbeit diskutieren wir die Probleme allgemein, die mit der Bestimmung der Qualität nicht-pheromonaler Gerüche zusammenhängen, außerdem formulieren wir Forschungsaufgaben, die zur Beantwortung ungelöster Fragen der olfaktorischen Codierung bei der Biene geleistet werden müssen.

sensorische Neurone / olfaktorische Codierung / antennale Plakoden / Elektrophysiologie (olfaktorische Sinneszellen)

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