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Wayne M. Getz · R. Patrick Akers

Response of American cockroach (*Periplaneta americana*) olfactory receptors to selected alcohol odorants and their binary combinations

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Abstract The cockroach is known to possess several morphologically distinct types of sensilla on its antenna, each of which contain a couple or a few receptor cells that respond to an array of compounds. We recorded the response of cells exclusively from one type of sensillum to evaluate the variation in the response of the cells in these sensilla to three closely related alcohols and their binary mixtures. Our results indicate that cells within the class of those responsive to aliphatic alcohols are otherwise variable in their response to particular aliphatic alcohols and not easily classifiable into subclasses. They also indicate that patterns of responses among cells are not robust with respect to concentration. Finally, a considerable level of inhibition is indicated in the response of the receptor cells to binary mixtures compared with the response to pure odorants. The data suggest that discrimination of alcohols (and other odorants of general but not special significance) by the cockroach cannot be understood simply in terms of labeled lines or linear filters.

Key words Olfaction · Odor discrimination · Mixture perception

Introduction

The American cockroach, *Periplaneta americana,* is one of the few insects that has been extensively studied in the context of both general and pheromonal olfactory systems at both the peripheral and central levels (reviews: Masson and Mustaparta 1990; Smith and Getz 1994). The male American cockroach has two specialist types of olfactory receptors, each responding to one of the two female pheromone components, periplanone A and periplanone B (Sass 1983). Sass (1976, 1978) provided the first systematic classification of generalist peripheral olfactory receptor types in males and females based on their sensitivity to a selected group of odorants and food odors. This was followed by a comprehensive study by Selzer (1984) on the specificities of American cockroach olfactory receptor cells to a range of alcohols, aldehydes, carboxylic acids, esters, ketones, and terpenes. More recently, Fujimura et al. (1991) identified eight major groupings of generalist olfactory receptor cells in the American cockroach based on the morphology of the antennal sensilla in which these cells are located and on the response of these cells to a range of fifty odorants of the types considered by Selzer (1984). These studies indicate that although some categorization of American cockroach generalist olfactory receptor cells into response types or classes is possible, considerable overlap exists among classes and some receptor cells defy classification.

The cockroach, however, is known to have several different morphological types of sensilla on its antenna (reviews Fujimura et al. 1991). According to Fujimura et al. (1991), only type S-I sensilla (single-walled hair-like sensilla 8–12 μm long) have receptor cells that respond to *n*-hexanol and *n*-heptanol. Within this group of sensilla, Fujimura et al. (1991) identified receptor cell types I, II, and III, based on dividing individual cell response levels to specific stimuli into one of four grades. Type I receptor cells were most sensitive to *n*-pentanol, but responded to *n*-hexanol. Type II receptor cells were most sensitive to *n*-hexanol, but responded to *n*-heptanol. Type III receptor cells were most sensitive to *n*-octanol and *n*-nonanol, but responded well to both *n*-heptanol and *n*-hexanol.

Since the broad tuning properties of cockroach peripheral olfactory receptor cells have already been explored in considerable depth (Sass 1976, 1978; Selzer 1984; Fujimura et al. 1991), we decided to focus on the finer discrimination properties of the neurons in the type

W.M. Getz (✉)
Department of Environmental Science, Policy & Management
University of California, Berkeley, CA 94720-3112, USA

R.P. Akers
California Department of Pesticide Regulation
1020 N St., Sacramento, CA 95814, USA

S-I sensilla. By selecting cells the respond to *n*-hexanol and *n*-heptanol, we know that we have receptor cells that are found only in type S-I sensilla. To focus on the abilities of populations of these cells to discriminate among similar compounds, we selected two other closely related aliphatic alcohols and the binary mixtures of the three compounds in question. In particular, we selected the three C6 aliphatic alcohols, 1-hexanol, 2-hexanol, and 2-E-hexen-1-ol, the first and third of which are known to be components of green leaf odor (Visser 1986; Olías et al. 1993). Since C6 compounds are generally ubiquitous in biological material, and cockroaches are generalist omnivores, one might expect a priori that they cockroaches are able to discriminate among these compounds.

In our study, we first evaluated how receptors responding to a mixture of these three components respond to the individual components themselves. We then analysed how the relative response to these three components may vary with concentration. Further, for the sake of comparison, we investigated the response of a second group of receptors to the parallel C7 set of odorants, 1-heptanol, 2-heptanol, and 2-E-hepten-1-ol. Unlike the first and third components of the C6 set of alcohols, none of these components are known to be ubiquitously associated with plant material. Thus, it is possible that receptors sensitive to the C7 mixture are less able to discriminate among components than the receptors sensitive to the C6 mixture are able to discriminate among the components of the C6 mixture. Whatever the outcome of this C6-C7 comparison, however, any differences or lack thereof provide some insights into how individual receptors may specialize to help an ecological generalist, such as the American cockroach, discriminate among a number of similar environmental (as opposed to pheromonal) odors.

Materials and methods

Data collection

Our recording methods are similar to those used previously on honey bees, roaches and other insects (O'Connell 1975; Sass 1978; Selzer 1984; Fujimura et al. 1991; Akers and Getz 1992, 1993) and our stimulation methods closely follow those used by O'Connell and others (O'Connell 1975; Grant and O'Connell 1986; Akers and O'Connell 1988; Akers and Getz 1992, 1993).

Only female roaches purchased as adults from Ward's (Rochester, New York) and fed on Ward's Cockroach Food were used since we did not want to confound our results through sexual differences that are known to exist in insect olfaction. The roach was attached to a custom-made stage by first removing its wings and legs and then laying it on its dorsum onto a piece of double-sided carpet tape. Narrow strips of duct tape were placed across the thorax and abdomen. Finally, its head was attached to the stage with low-melting point wax. An antenna was fixed to a glass slide on the stage by laying it down on a piece of double-sided clear tape and by placing narrow strips of clear tape across it at intervals of every 3–5 mm (Fujimura et al. 1991). Another droplet of low-melting-point wax was placed along one side of one or two flagellar subsegments, near the distal end of the antenna. This provided support when the reference electrode was inserted into the antenna. Once the roach was prepared, the stage was positioned under the objective of the microscope.

The microscope (Nikon Labophot, with 10× and 40× long-working distance objectives) rode on an x-y translation stage on the base plate of the setup, while the preparation remained fixed with respect to the base plate. Epillumination with a fiber optic illuminator was preferable for this preparation.

Electrodes were fashioned from 125 μm tungsten wire, and were electrolytically sharpened to less than 1 μm. The reference electrode was placed in the tip of the antenna. Recordings generally began near the base of the flagellum and proceeded towards the tip. All the recordings were taken from sensilla basiconica (delicate sensory pegs 1–3 μm in diameter and 8–20 μm long), but we did not distinguish among the morphological sub-types (Fujimura et al. 1991).

Signals were amplified differentially, with a bandpass of 300–3000 Hz. Control of stimulation of the antenna was integrated with A/D data acquisition on a microcomputer. Two air streams were aligned facing each other on either side of the antenna. Both streams came from a cylinder of compressed breathing quality air, filtered through DrieRite and a 5-Å molecular sieve (gas purifier Model L68GP, Cole-Parmer). The air was not humidified after passing through the purifier. Sensilla did not measurably respond to turning the air streams on and off.

The clean air stream, or purge, flowed continuously over the target subsegment at ca. 210 ml min^{-1}, except during stimulation. The stimulus airstream was ca. 60 ml min^{-1}. Stimuli were delivered by flowing the stimulus airstream through a cartridge containing an odor in 1 μl of mineral oil on a 7 × 35-mm slip of Whatman No. 1 filter paper. The cartridge was a 5 cm length of 3/32 in. ID Teflon tubing, with a press-on female Luer fitting on one end. The opening of a cartridge was positioned 4–5 mm from the antenna. In all stimulations, the stimulus line was first turned on for 0.5 s with the purge still on (Grant and O'Connell 1986). This flushed the stimulus cartridge with approximately two volumes of clean air. As our recordings, and trials with smoke laiden air indicated, the purge stream effectively kept the released odorant from reaching the antenna. After this 0.5-s period, the purge stream was immediately turned off for 0.5 s, allowing the odorant to reach and stimulate the antenna. At the end of 0.5 s, the purge was turned back on and the stimulus stream was turned off. Stimuli were generally presented at 1- to 2-min intervals. Data obtained from experiments with pulsed stimuli (Lemon and Getz, in press) indicate that this is sufficient time to permit complete disadaptation of receptors.

Two experiments with mixtures of odorants were run in parallel. One experiment used the hexyl compounds 1-hexanol (6:1ol), 2-hexanol (6:2ol), and 2-E-hexen-1-ol (2E6ol) while the other experiment used the analogous heptyl compounds 1-heptanol (7:1ol), 2-heptanol (7:2ol), and 2-E-hepten-1-ol (2E7ol) (all odorants where obtained from Aldrich Chemical Co., except for 2E7ol which was obtained from Lancaster, and they varied in purity from 97 to 99%). A sensillum was first presented a search stimulus comprised of all six compounds in a mixture at 10 μg μl^{-1} each. If there was a noticeable response to the search stimulus, the sensillum was presented with two more search stimuli. One stimulus contained the hexyl compounds at 10 μg μl^{-1} each, and the other contained the heptyl compounds at 10 μg μl^{-1} each. The sensillum was then presented with a mixture series corresponding to the search stimulus that appeared to produce the stronger of the two responses from the sensillum as a whole. We made an attempt to keep the number of replicates for each experiment roughly equal as the data were collected. This sometimes meant that we passed over a responsive sensillum if it responded to a series that was several replicates ahead.

Once it had been determined whether the sensillum would be tested with either the hexyl or the heptyl compounds, it was then exposed to the appropriate three odorants at several different concentrations (typically the first or last four of the five concentrations 0.1, 1, 10, 100, and 600 μg μl^{-1}) and their three binary mixtures at matched concentrations (each component at half of the pure odorant stimuli to which the binary stimulus is matched). Note the highest dosage was 600 rather than 1000 μg μl^{-1} because the latter concentration levels generally could not be achieved in

liquid phase. We estimated the dosage at which to begin the series from the response to the search stimuli, with the goal of obtaining an initial response just at or above background. As became apparent after we had analyzed our results, however, this approach does not account for the fact that the response (spiking frequency) of the neurons to mixtures is generally lower than the response of the same neurons to comparable concentrations of the pure odorant components of these mixtures. Dosage progressed from low to high, but stimuli were presented at random within a dosage level. A blank control stimulus (1 μl mineral oil alone) was given at the start of each series and after approximately every sixth stimulus. Data were collected from twenty-five sensilla in each experiment.

The spikes in the record from each sensillum basiconicum were digitized and sorted by shape using the software package SAPID Tools (Smith et al. 1990), usually into two classes. Each class unit ideally represents the activity of a single neuron, although some errors in sorting spikes are bound to occur. Each basiconicum of the roach is innervated by one to four neurons (Schaller 1978; Fujimura et al. 1991). We identified from one to three shape classes of spikes in our data, but the most common number of classes was two. Potential classification errors were minimized by examining distributions of height-width plots of classified spikes and manually reclassifying (using a facility within SAPID Tools) any spikes that appeared to be misclassified.

Data analysis

The data analyzed below are all based on the number of spikes in each shape class that we counted over the 0.5-s odorant stimulus interval which occurred in the middle of the 1.5-s recording interval. Note that we explicitly identified each of the one to three spike shape classes associated with the 20–35 spike trains that we obtained for a particular sensillum so that we knew the response of each cell to all of the different stimuli presented to the sensillum. For clarity of exposition, let x_{ij} represent the number of spikes that receptor cell i fired over the 0.5-s stimulus interval in response to the jth stimulus (each cell received from 20 to 35 stimuli – six odorants at three to five concentration levels plus several pure mineral oil controls). Also, we ensured through the comparison of height-width plots, available in SAPID Tools, that each of these shape classes was consistent across all spike trains recorded from the same sensillum. Thus the vector $x_i = (x_{i1}, x_{i2}, \ldots, x_{i12})'$ represents the "absolute response rate" profile of the ith receptor cell over a set of m stimuli $S_j, j = 1, \ldots, m$. The "net response rate" profiles are $y_{ij} = x_{ij} - d_i$, where d_i is the average response of the ith cell to the two to four pure mineral oil control stimuli (this response is interpreted as a background firing rate). From this we obtain a set of corrected response data $y_i = (y_{i1}, y_{i2}, \ldots, y_{i12})'$ over the same set of n olfactory receptor cells (i.e., for $i = 1, \ldots, n$). Finally, the "normalized net response rate" profiles $z_i = (z_{i1}, z_{i2}, \ldots, z_{i12})'$ are obtained using the formula $z_{ij} = y_{ij} \Big/ \sqrt{\sum_{k=1}^{m} y_{ik}}$, where z_i now is a representation of the "tuning spectrum" of the i receptor cell relative to the set of m stimuli in question (Akers and Getz 1992). In all cases, we normalized over six odor stimuli $S_j(c_l), j = 1, \ldots, 6$, delivered at the same concentration c_l, where $l = 1, 2, 3, 4$, or 5 and $c_1 = 0.1, c_1 = 0.1, c_2 = 1\ \mu g\ \mu l^{-1}$, $c_3 = 10\ \mu g\ \mu 1^{-1}$, $c_4 = 100\ \mu g\ \mu l^{-1}$, and $c_5 = 600\ \mu g\ \mu l^{-1}$. In the Results section, we indicate whether the results under discussion relate to the absolute response data $x_i(c_l)$, the corrected response data $y_i(c_l)$, or the normalized response data $z_i(c_l)$.

Results

Concentration response profiles

We obtained recordings from 25 sensilla using our heptyl suite of stimuli and from a second set of 25 sensilla using our hexyl suite of stimuli. Although the two sets of data were collected from completely different animals, in both cases, recordings from three of the sensilla were from one animal, six animals were used to obtain two recordings from each, and one recording was obtained from ten other animals. Further, when more than one recording was made from the same animal, the sensilla were from different segments of the antenna. Since the 25 sensilla used in each case were from 17 different animals and no evidence exists to link the firing characteristics of different sensilla on the same animal, the assumption we make that the data obtained from each sensilla are statistically independent of the data from all the other sensilla appears to be reasonable. In approximately 15%, 70% and 15% of the sensilla, we could respectively identify one, two, and three classes of spikes. We presume, as is generally done, that each spike class represents the activity of a distinct cell, although it is always possible in cases where we observe only one spike class that some of these spike trains could represent the activity of two cells generating similarly shaped spikes. In none of these cases, however, did we observe the partial superposition of two spikes that can only be produced by different cells firing within a couple of milliseconds of each other. Also, none of our single spike classes had activity rates that lead us to believe we were observing the joint activity of two cells. A complicating factor, however, is that the shape of the spikes (especially the amplitude) produced by a particular cell may change during high frequency firing. We tried to account for this when examining traces to make manual corrections. Although some of our results may be sensitive to misclassifications, others, such as the detection of inhibition in response to binary odor stimuli, are not.

Most of the receptor cells responded to all six stimuli with spiking rates that increased with concentration (e.g., cell 1.1 and 48.1 in Fig. 1). A few, however, responded to one odorant much more strongly at some of the concentrations than the other odorants (e.g., cell 4.2 in Fig. 1). A few unusual cells were present such as cell 9.2, which responded most strongly at the lowest concentration and then somewhat moderately at the highest concentration, and cell 49.2 (Fig. 1), which had an unusually high response level to the control that was strongly inhibited at moderate to high stimulus concentrations. Our method of selecting cells, however, was biased against detecting cells of type 9.2 (Fig. 1), since such cells respond weakly to the test stimulus and, as described in the methods section, may be rejected as inactive and hence not subjected to the full battery of stimuli. Thus, it is difficult for us to assess how rare cells of type 9.2 might really be. Another relatively small group of cells, such as 17.1 and 33.1 (Fig. 1) had response profiles almost independent of concentration: they behaved like presence/absence detectors. A few cells such as 10.2 and 39.2, had low spiking rates that varied around the control levels (note the considerable differences in the vertical scale from graph to graph in Fig. 1). These cells were excluded from further analysis since their output represented noise around the control level.

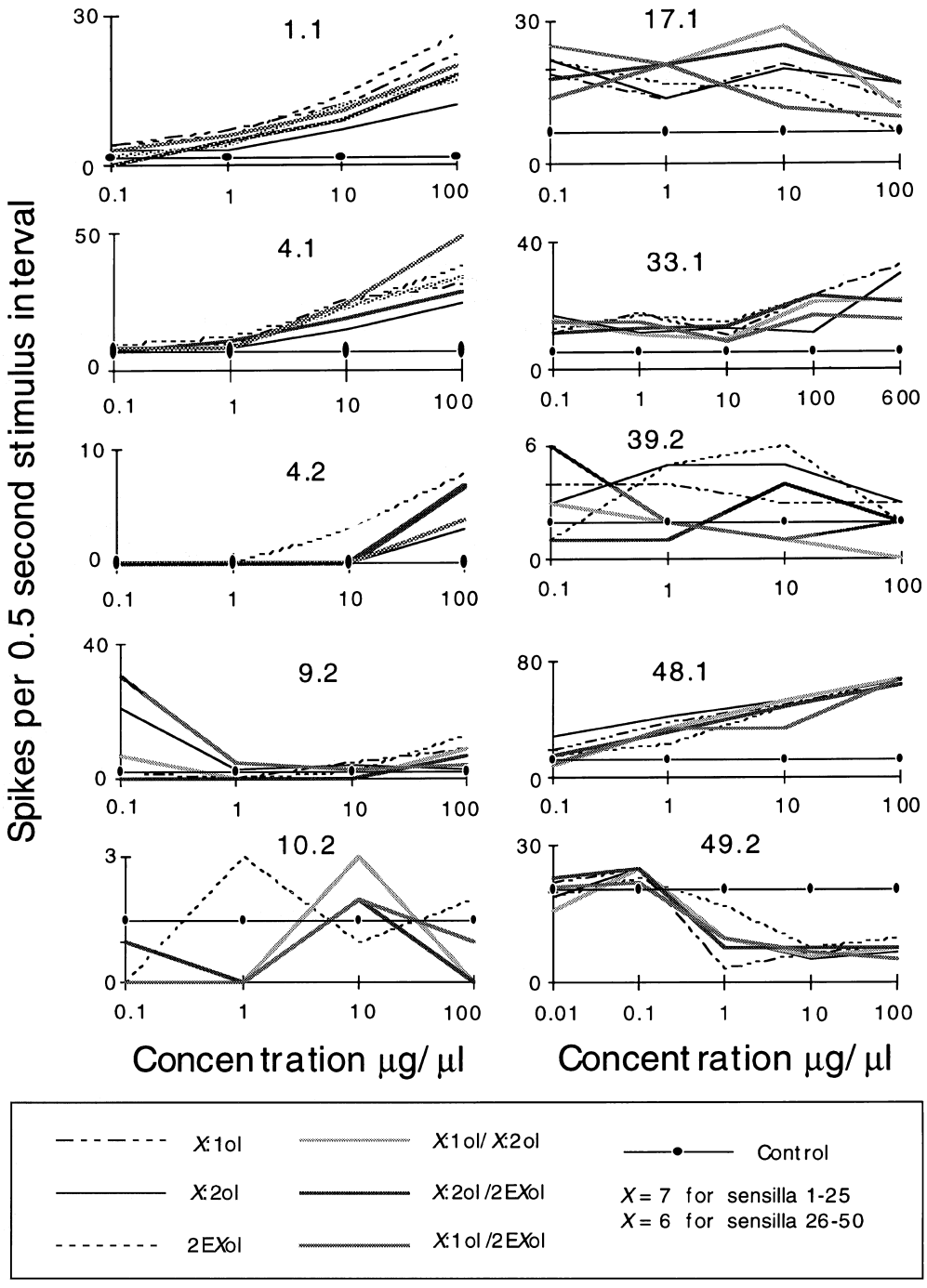

Fig. 1 The spiking rates (number of spikes per 0.5-s interval – note that each vertical scale is different) of a selection of 10 cells out of a population of 100 cells (from 50 different sensilla) used in this study, are plotted as a function of stimulus concentration

The response data from the remaining cells were used in a number of different types of analyses.

Group analyses

The software package SYSTAT (v 5.2.1 for the Macintosh; Evanston, Il) was used to obtain Pearson's product moment correlations for the response of the two groups of cells (those stimulated with the hexyl and those with heptyl compounds) at each of the five concentrations. For each concentration, $l = 1, .., 5$, we used the corrected response data $y_i(c_l)$ across each group of cells i (note that these groups are not the same for each concentration particularly the lowest and highest concentration where, as described in Materials and methods, we collected data from fewer cells than at the intermediate concentration) to obtain correlations in the response of these cells to different pairs of stimuli. Since we had six stimuli, 15 correlations were obtained (e.g., correlations in the response of a group of cells to 1-heptanol and 2-heptanol or to 1-heptanol and a blend of 1-hep-

tanol and 2-E-hepten-1-ol are 2 of the 15 correlations). The average and ranges of these correlations for each set of stimuli are given in Table 1.

For the heptyl stimuli these correlations increase significantly (see Table 1) with concentration, and the increases are significant in going from concentrations 1 to 10, 10 to 100, and 100 to 600 μg μl^{-1}. For the hexyl stimuli, on the other hand, the correlations rise with concentration up to 100 μg μl^{-1} and then significantly ($P < 0.001$-see Table 1) drop when the concentration increases from 100 to 600 μg μl^{-1}.

We used Ward's minimum variance method of cluster analysis, as implemented on the software package SYSTAT [v 5.2.1 for the Macintosh, Evanston, Il – also see Romesburg (1990)], on the normalized data $z_i(c_l)$ (which is suitable for characterizing the tuning spectrum of each cell since the differences in the magnitude of the responses of each cell are removed by normalization) to assess the characteristics of our group of cells in categorizing the different odor stimuli at the various concentrations (Fig. 2).

We then went on to perform principal components analyses on these data. In this analysis, since we were interested in investigating whether differences at a group level existed in the responses of the largest spiking and the second largest spiking cell in each the

Table 1 Average correlations in the "net response rate" of cells across all pairs of odorants

Stimulus Group	Concentration (μg μl^{-1})	Sample Size	Average	Range	Significance[a]
Heptyl	0.1	18	0.529	[−0.03,0.83]	
	1	35	0.665	[0.51,0.83]	NS
	10	37	0.735	[0.40,0.93]	$P < 0.05$
	100	41	0.849	[0.69,0.91]	$P < 0.01$
	600	21	0.883	[0.82,0.94]	$P < 0.05$
Hexyl	0.1	22	0.724	[0.52,0.88]	
	1	41	0.839	[0.66,0.94]	NS
	10	44	0.918	[0.81,0.98]	$P < 0.05$
	100	45	0.921	[0.82,0.97]	NS
	600	23	0.695	[0.48,0.83]	$P < 0.001$

[a] The hypothesis that the set of correlations used to obtain the average is not significantly different for the concentration on the indicated line compared with the concentration on the line immediately above was tested using the sign test of Dixon and Mood (i.e., the 15 types of correlations at each concentration where matched according to the two stimuli used to obtain the correlation and two-sided probabilities evaluated for obtaining x out of 15 larger or smaller)

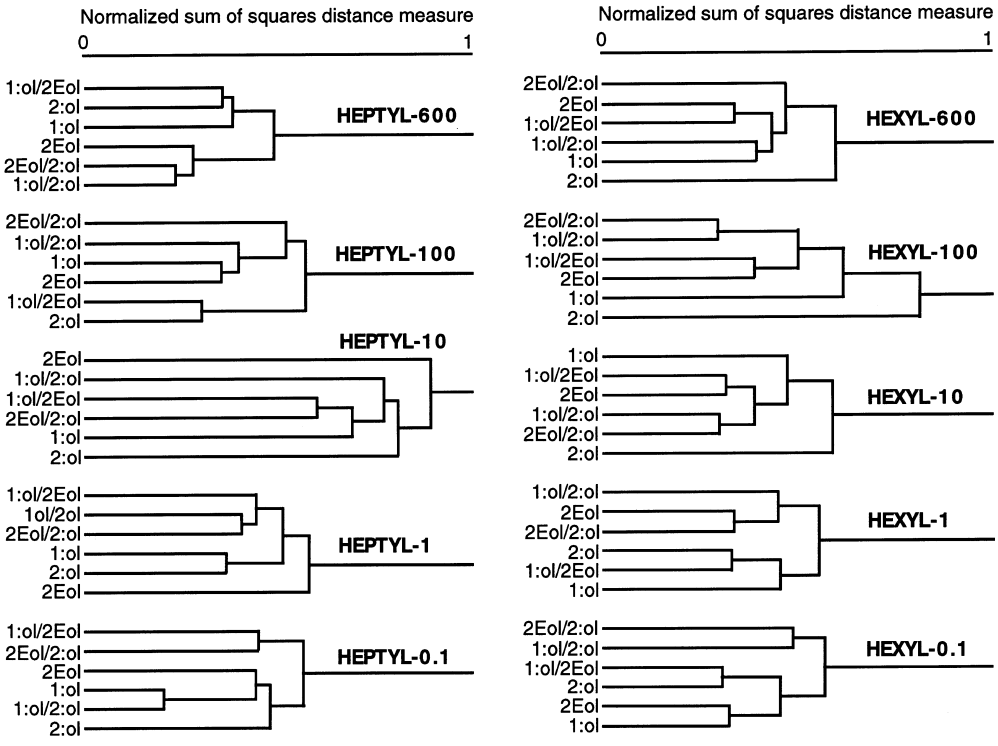

Fig. 2 A Ward minimum variance cluster analysis of Euclidean distance among the normalized response data of all cells to the hexyl and heptyl stimuli at the indicated concentrations of 0.1–600 μg μl^{-1}

sensilla, we only included data from pairs of cells that were matched by sensillum. Here we investigated the data only at concentrations 1, 10 and 100 μg μl^{-1} for each of the suites of stimuli because the number of matched pairs at the lowest and highest concentrations were too small to justify a principal components analysis. We plotted each cell on the space spanned by the first two principal components (Fig. 3). The amount of variance explained by these two components varied between 60 and 73% (indicated in parenthesis on axis labels in Fig. 3).

Binary mixtures

For any two odorants O_1 and O_2 at concentration c_l, let $x_{i1}(c_l)$ and $x_{i2}(c_l)$, respectively, denote the absolute response of the ith cell to these two pure odorants, and let $x_{i12}(c_l)$ denote the response of this same cell to a $0.5c_l$: $0.5c_l$ mixture of these two odorants. Then, under the assumption that any non-linearities in the response of the receptor cells are due purely to the kinetics of one or more odorants differentially competing for one or more membrane receptor sites, and that the spiking rate of the

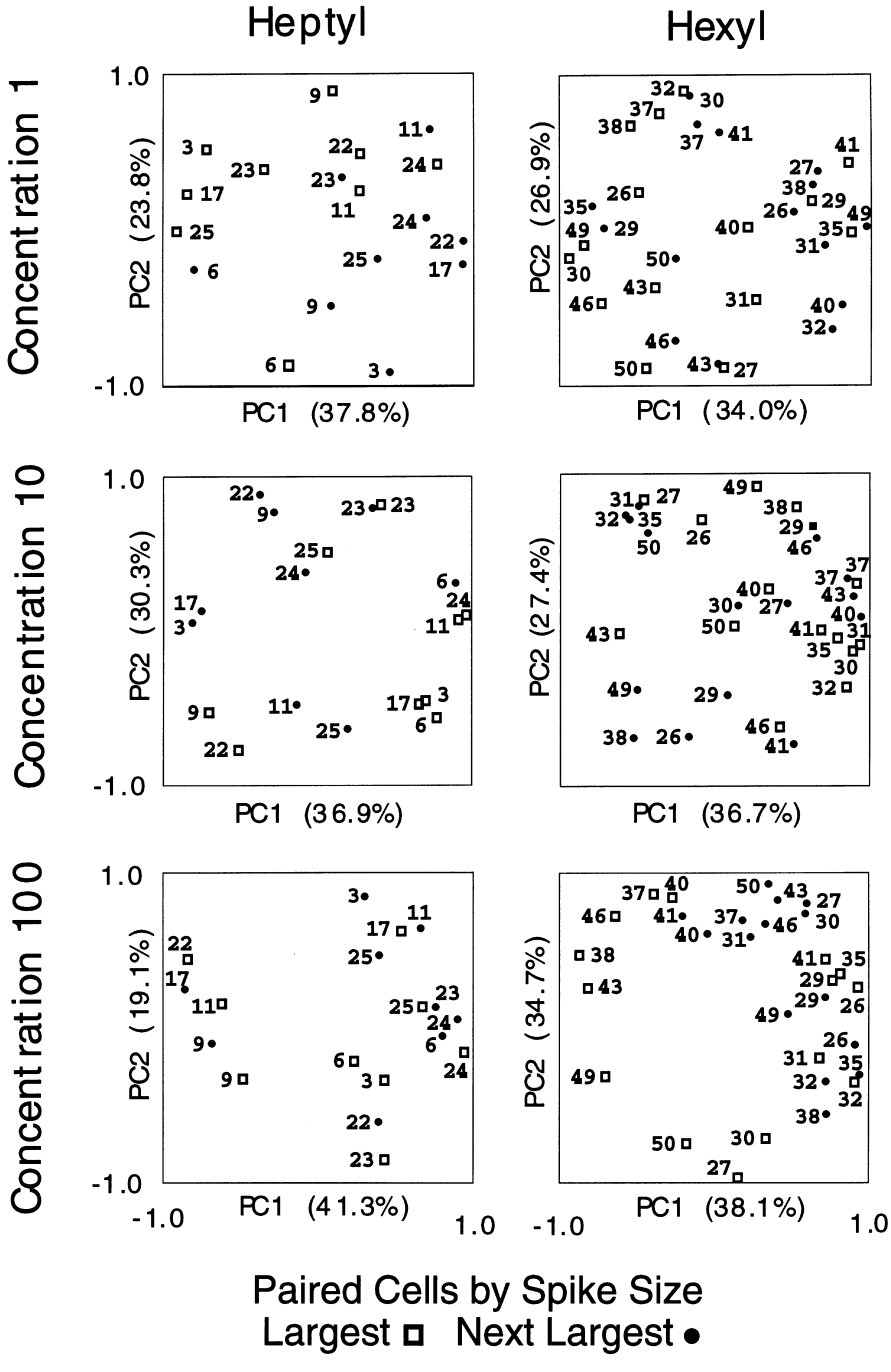

Fig. 3 The response of selected matched pairs of the largest and second largest spike classes (cell classes) are plotted against the first two principal components (percentage of explained variance indicated in parenthesis) obtained from a principal component analysis at the labeled concentrations (in μg μl^{-1})

cell is an increasing concave function (positive first and negative second derivatives) of the number of activated receptors sites, the response (i.e., spiking rate) to the binary mixture should lie between the average of the responses to the two pure odorants and the maximum of these two responses – that is (see Getz and Akers 1995)

$$\frac{x_{i1}(c_1) + x_{i2}(c_1)}{2} \leq x_{il2}(c_1) \leq \max\{x_{il}(c_1), x_{i2}(c_1)\}. \qquad (1)$$

If this inequality holds for a particular binary stimulus and components at the matched concentrations, and for a given cell the response is scored as "between." On the other hand, if either the left or the right hand inequality in (1) is respectively violated, the response to the concentration-matched mixture is scored as "below" or "above." A significant number of "below" or "above" violations across a population of cells for the binary mixture in question respectively reflects inhibitory or synergistic processes that are independent of any type of concentrations effects or direct competition between odorant molecules for membrane receptor sites.

Such inhibitory type processes were identified for every tested combination of odorants at every concentration used (Table 2). In contrast, no significant synergistic effects were detectable using the inequality test above in any of the odorant combinations used. No consistent pattern in the proportion of inhibitory identifications is evident across concentration or with respect to the functional type of the odor stimuli. However, the proportion of inhibitory identifications is significantly higher in the hexyl set of data than in the heptyl set of data. Specifically, the total proportion of cells that exhibited inhibition in the hexyl data set (64.2%) is significantly higher than the proportion for the heptyl data set (53.4%) at the $P < 0.001$ level (G-test).

Table 2 Percentage of responses to mixtures categorized according to inequality (2)

Odorant Pair	Conc. $\mu\gamma/\mu\lambda$	Sample Size (N)	Response to Mixture (%) Below	Between	Above
6:1ol/6:2ol	0.1	21	61.9[b]	16.6	21.4
	1	40	54.6[b]	15.8	29.6
	10	44	75.8[c]	11.0	13.3
	100	45	67.8[c]	15.6	16.7
	600	23	60.9[b]	13.0	26.1
	all	173	65.1[c]([a])	14.3	20.6
6:2ol/2E6ol	0.1	21	58.0[a]	17.4	24.6
	1	40	58.8[c]	11.2	30.0
	10	44	70.1[c]	14.4	15.5
	100	45	75.6[c]	8.9	15.6
	600	23	60.9[b]	17.4	21.7
	all	173	66.2[c]([a])	13.0	20.8
6:1ol/2E6ol	0.1	21	56.4[a]	8.7	34.9
	1	40	41.3	27.5	31.2
	10	44	74.3[c]	7.2	18.6
	100	45	65.2[c]	13.0	21.8
	600	23	67.4[c]	19.6	13.0
	all	173	61.1[c] (c)	15.2	23.6
Hexyl total		519	64.2[c] ([d])	14.2	21.7
7:1ol/7:2ol	0.1	18	70.4[c]	14.8	14.8
	1	35	47.2	18.6	34.3
	10	37	41.4	23.9	34.7
	100	41	53.3[b]	26.4	20.3
	600	21	59.5[b]	28.6	11.9
	all	152	51.9[c]([a])	22.9	25.2
7:2ol/2E7ol	0.1	18	57.4[a]	10.2	32.4
	1	35	53.3[b]	14.8	31.9
	10	37	64.9[c]	14.8	20.3
	100	41	48.0[a]	29.7	22.4
	600	21	52.4	14.3	33.3
	all	152	55.1[c]([b])	18.2	26.8
7:1ol/2E7ol	0.1	18	38.9	16.7	44.4
	1	35	60.0[b]	8.6	31.4
	10	37	52.7[b]	18.9	28.4
	100	41	51.2[a]	22.0	26.8
	600	21	59.5[b]	21.4	19.0
	all	152	53.3[c](c)	17.4	29.3
Heptyl total		456	53.4[c]([d])	19.5	27.1

[a,b,c] (G-test) indicate signif. at $P < 0.05$, 0.01, 0.001. The binomial distr. with param. 1/3 is used to test deviations for the responses. A G-test is used to test differences on entries followed by the same letter in parantheses

Discussion

As pointed out in the Introduction, our selection of odors implies that the receptor cells we stimulated are found only in type S-I sensilla (Fujimura et al. 1991). Because our sampling protocol implies that the data do not come from sensilla sampled at random, our analysis of the tuning properties of these cells is not a complete representation of the filtering characteristics of the olfactory periphery with respect to the odors used. Thus, our approach underestimates rather than overestimates the diversity or richness of the filtering properties of the periphery. Further, the comparison made between the responses to the C6 and the C7 compounds remains valid, since our sampling protocol treated this two sets of stimuli in the same way.

The results in Figs. 2 and 3 indicate that the pattern of responses of these receptor cells are much more complicated than is suggested by a classification of these receptor cells into three – or even several – types: a few types may fall out when considering responses to a wide array of compounds (Selzer 1984; Fujimura et al. 1991), but our results indicate that, at least within the types of cells that respond to C6 and C7 aliphatic alcohols, an array of cells exist with response properties that vary considerably from cell to cell.

First, the average correlation among the group of cells tested with the heptyl suite of stimuli (Table 1) is moderate (average value of 0.529, range of −0.03 to 0.83) at the lowest concentration (0.1 $\mu g\ \mu l^{-1}$), but increases significantly as the concentration increases from 1 to 10 ($P < 0.05$), 10 to 100 ($P < 0.01$), and 100 to 600 ($P < 0.05$) $\mu g\ \mu l^{-1}$ (at the highest concentration the average value is 0.883 with a range of 0.82–0.94). Note, this increase with concentration is expected whenever cells are specialists at low concentrations and generalists at higher concentrations (e.g., Fig. 1, cell 4.2 responds to only one of the stimuli at concentrations of 10 $\mu g\ \mu l^{-1}$, but responds to all the stimuli at concentrations of 100 $\mu g\ \mu l^{-1}$). The group of cells tested with the hexyl suite of stimuli (Table 1) deviates from this pattern of increasing correlations with concentration because a highly significant ($P < 0.001$) drop in the average value of the correlation is evident when the concentration increases from 100 to 600 $\mu g\ \mu l^{-1}$. The reason for this drop is unclear, but it could be due to the fact that, as discussed in the Results section (Table 2), the hexyl group is significantly ($P < 0.001$) more inhibited in its response to mixtures versus pure odorants than the heptyl group.

Second, as evidenced by the classification patterns in Fig. 2, neither of the two groups of receptors (those tested with hexyl and those tested with heptyl compounds) produce strong associations between component odorants and related mixtures. Further, in both cases, no consistent patterns of association emerge across the ranges of concentrations.

Third, the tuning spectra among both groups of receptor cells is such that no clear subgroupings of cells responding to our C6 or C7 compounds exists. This latter finding agrees with the response correlations that Selzer (1984) identified among cockroach olfactory receptor cells stimulated with a diverse array of 25 straight-chained, branched, and cyclic odorants of various moieties (alcohols, aldehydes, ketones, acids, and amines).

A further difference between the heptyl and hexyl sets of data is that the heptyl data indicate some separation of largest and next largest spike classes in the same sensillum at a concentration of 1 $\mu g\ \mu l^{-1}$. At the highest concentration, however, no separation between the largest and next largest spike classes is evident (e.g., the cluster 3, 17, 23, and 25 of largest spike classes and the cluster 9, 17, 22, 24, 25 of second largest spike classes), even though at this concentration we get the tightest clustering of all cells into distinct groups (four in this case – cf. Fig. 3, bottom left panel). For the hexyl data, the situation is reversed in that we get the best separation between the largest and next largest spike classes at 100 rather than 1 $\mu g\ \mu l^{-1}$ (note the cluster of second largest spike classes in the upper right-hand side and largest spike classes in the upper left hand side of the hexyl plot at 100 $\mu g\ \mu l^{-1}$). Visual inspection indicates that as in the heptyl case, however, we still get the tightest clustering at concentration 100 $\mu g\ \mu l^{-1}$. These results indicate that no obvious response classification scheme exists for the largest and next largest spike classes in the same sensillum and that no special relationship appears to exists between these two classes of cells when concentration is taken into account.

Although the olfactory neurons do not fall into neat categories, sufficient similarities in the responses exist so that some diffuse clustering of cells does occur (Fig. 3). If similarly "tuned" cells converge on the same glomerulus in the antennal lobe of the insect [note that in insects most olfactory cells appear to converge on only one glomerulus – for a review of the neuroanatomy and physiology of the insect olfactory system see Masson and Mustaparta (1990)], then it is clear that the input received by neighboring glomeruli can be quite different. Presumably, it is such differences at the glomerular level that ultimately account for the odor discrimination abilities of the insect olfactory system.

It still remains a puzzle, however, how the highly non-linear responses of insect olfactory receptors to changes in concentration and components of mixtures are compatible with the process of identifying the quality of an odor stimulus across a range of concentrations. Our difficulties in solving this puzzle arise, in part, from the preconceptions we have on how olfaction ought to work, based on our knowledge of the specialized olfactory cells and pathways insects use to detect and perceive pheromones. They also arise, in part, from oversimplified mathematical concepts of how the antennal lobe might map olfactory input onto projection neuron output in invertebrate systems (Getz and Chapman 1987) or onto mitral cell output in vertebrate systems (Schild 1988).

The observed non-linearities, rather than degrading olfactory perception, might actually enhance it. For example, the fact that some cells respond most strongly at low concentrations (e.g., cells 9.2 and 49.2 in Fig. 1) and others at high (e.g., cells 1.1. and 48.1 in Fig. 1) may enhance the concentration range over which the quality of odors can be reliably detected. Also, the prevalence of inhibition in binary mixtures may enhance the performance of the system in assigning a quality to a mixture that is distinct from the components of the mixture. Further, inhibition in mixtures may help to refine the ability of the insect olfactory system to discriminate between mixtures made up from the same components, but in different proportions.

The most difficult aspect to explain regarding observed non-linearities at the receptor population level, however, is the lack of some observable invariant in the firing patterns for stimuli of the same odor quality at different concentrations. If this invariant existed, it would be interpreted as a representation of the odor quality of the different stimuli. From Figs. 2 and 3 it is clear that relationships among cells change quite markedly with concentration. Bearing in mind that the output from the antennal lobe is itself input into higher processing neuropil structures, namely the mushroom bodies of the insect brain, it is not necessary that a stable pattern exists across concentrations in order for stimulus quality to be assessed independently of concentration. It is only necessary that repeatable sets of patterns be generated for stimuli that are qualitatively the same. Then the mushroom bodies can learn to associate the whole set of patterns with a particular quality (Malaka et al. 1996).

During the past couple of decades, our ideas regarding olfactory coding have moved from a concept of labeled lines, which may still be appropriate for understanding pheromonal perception, to a concept of across fibre patterns (reviews: Masson and Mustaparta 1990; Smith and Getz 1994). This latter concept is no longer adequate when one considers olfactory coding at the peripheral level of general plant odorants such as C6 aliphatic alcohols when concentrations vary with time, and, as discussed elsewhere (Getz and Akers, 1997), when the temporal characteristics of olfactory receptor responses are taken into account.

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