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Coding properties of peak and average response rates in American cockroach olfactory sensory cells

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Abstract

The response of phasic neurons is often measured in terms of average spiking rates over arbitrarily selected time intervals. Averages taken over inappropriate intervals may severely reduce the information content of data, as we show here using response data from female American cockroach peripheral olfactory cells. We demonstrate that a 100-ms period around the peak response contains the best information for discriminating among odors at moderate to high concentrations. Further, the 100-ms post-peak response period contains the best information at low concentrations, as well as in situations where it is important to minimize errors in misidentifying the quality of an odor. Averaging the data over the full 0.5 s stimulus period degrades the odor separation qualities of the data.

Keywords: Insect olfaction; Monte Carlo simulation; Sensillum basiconica

1. Introduction

Our current understanding of olfactory coding in the brains of insects is embryonic and typified by rudimentary notions of information coded by 'labeled lines' or, more generally, 'across fiber patterns' (Smith and Getz, 1994). These notions usually ignore temporal aspects of the response of olfactory sensory neurons located in sensilla on the antennae, although olfactory coding in insects is known to have a strong temporal component (Marion-Poll and Tobin, 1992; Laurent and Davidowitz, 1994). These notions also ignore nonlin-

earities that arise from the interplay of excitatory and inhibitory processes in the generation of the responses of olfactory sensory neurons (DeJong and Visser, 1988; Akers and Getz, 1993; Getz and Akers, 1995), as well as other non-linearities that invoke different patterns of response at different concentrations of the same stimulus (Getz and Akers, 1996). The effects of these non-linearities are compounded by the fact that the stimuli themselves are highly variable with respect to concentration in both space and time (Murlis et al., 1992).

In an individual adult insect, such as a bee or a roach, several tens of thousands of olfactory sensory neurons that originate in each of its two antennae and then project onto a hundred or so

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glomerular neuropil structures in the antennal lobe of the deutocerebrum on the ipsilateral side of the antenna from which they originate (Rospars, 1988; Masson and Mustaparta, 1990). The exact function of the glomeruli is unknown. Given the 100–1000-fold degree of convergence of sensory receptors onto glomeruli, however, and the fact that each receptor cell arborizes in only one glomerulus, it is reasonable to speculate that among other things, the glomeruli smooth out spatial and temporal heterogeneities in stimuli and integrate out some of the non-linearities. Each glomerulus sends its output via relay neurons that project to higher centers of the brain such as the mushroom bodies and lateral protocerebrum (Hammer and Menzel, 1995). One can further speculate that activity of these relay neurons is sufficiently smoothed, integrated, and contrasted among one another through the action of local, generally inhibitory, neurons that project among the different glomeruli (i.e. the intrinsic interneurons), to contain a stable representation of the quality of the input stimulus at the periphery. Here we will analyze our data in a way that takes cognizance of the convergent architecture of the glomerular components of the olfactory system, and explores the consequence of averaging data over different intervals of time.

2. Data collection

We have reported elsewhere on several aspects of the average response of individual cockroach (Getz and Akers, 1996) and honey bee (Akers and Getz, 1992, 1993) cells over 0.5-s stimulus intervals to suites of stimuli presented at different concentrations. The cockroach data were obtained from extracellular spike trains recorded using tungsten electrodes inserted at the base of olfactory sensilla on the antennae of the female American cockroach (*Periplaneta americana*). Our recording methods are similar to those used previously on honey bees, roaches, and other insects (Selzer, 1984; Fujimura et al., 1991; Akers and Getz, 1992, 1993).

Only female roaches were used to obtain the data reported here. All the recordings were taken from sensilla basiconica (delicate sensory pegs

1–3 mm in diameter and 8–20 mm long), but we did not distinguish among the morphological sub-types (Fujimura et al., 1991). All stimuli lasted for 0.5 s. Fifty sensilla, each exhibiting the activity of 1–3 (typically 2) olfactory sensory cells, were either stimulated with a suite of 6 ‘hexyl’ (1-hexanol, 2-hexanol, and 2-E-hexen-1-ol and their three binary combinations) or a suite of six ‘heptyl’ (1-heptanol, 2-heptanol, 2-E-hepten-1-ol and their three binary combinations) stimuli. The odorants were presented at concentrations 0.1, 1, 10, 100, and 600 $\mu\text{g}/\mu\text{l}$ for the pure odorants and 0.5:0.5 blends for the components of the binary blends so that the total concentrations for the blends were also 0.1, 1, 10, 100, and 600 mg/ml. The control was an odorant free 1 μl of mineral oil (for more details see Getz and Akers, 1996).

Approximately 20–30 stimuli were applied to each sensillum. The digitized recordings of the spike trains began 0.5 s prior to stimulation, continued for the 0.5 s during stimulation and then an additional 0.5 s after stimulation. The spikes in each of these 20–30 1.5-s records were digitized and sorted by shape using the software package SAPID Tools (Smith et al., 1990).

3. Temporal response and hypothetical glomeruli

The number of spikes in each of the 1.5-s long digitized spike train were sorted into 30, 50 ms time bins. For each of the five concentrations and each of the two suites of odors, the numbers of spikes in each of the 30 time bins were averaged across the populations of individual cells (from 19 to 46 cells in each of the populations, depending on the concentrations) and across the responses of these cells to the three pure odorants or the three binary mixtures. These mean values and associated standard errors were then plotted against time (Fig. 1). These plots reveal that activity of cells reaches a concentration dependent peak within 100 ms after the onset of the stimuli and then rapidly declines to levels less dependent on concentration. The spiking rate drops virtually to 0 within 50 ms of the stimulus being switched off. Further, the responses to the mixtures are slightly suppressed compared with the response to the pure odorants. This is consistent with the fact

that mixture inhibition is common in insect olfaction (DeJong and Visser, 1988; Akers and Getz, 1993; Getz and Akers, 1995). This suppression is most obvious at the lowest concentrations.

The response of cells to these stimuli is highly phasic (Fig. 1). Below we compare the coding efficiency of the data over the 'full' 500 ms, a 'peak' 50–150 ms, and a 'post-peak' 150–250 ms periods of stimulation. For each concentration, the response (i.e. average spiking rate) of each cell over a selected period of time to the six stimuli in question (three pure odorant and three binary mixtures for the hexyl or heptyl suites) represents a point in a 6-dimensional space, where the axes are the six stimuli. The direction of the vector connecting the origin to this point is a representation of how the cell is 'tuned' to respond to stimuli. Since similarly tuned cells often project to the same glomerulus in the antennal lobe of the insect brain (Hansson et al., 1993), we identified 2–4 groups of such cells using Ward's minimum variance clustering algorithm on the normalized (i.e. directional) data. Analyses were carried out on a Macintosh computer using SYSTAT 5.2.1 (SYSTAT Inc., Evanston, IL, 1992), where the actual number of groups in each case depended on how well the neurons clustered. For example, at the 100 $\mu\text{m}/\mu\text{g}$ concentration of the heptyl set of data, the response spectrums of the cells to the six stimuli in question (Fig. 2) clustered into four different groups for each of the three intervals of interest (full, peak, and post-peak).

The mean response of each cluster of cells, over the period of interest, to a particular one of the six stimuli represents a point in an m -dimensional vector space where m is the number of clusters (four in this case of the 100 $\mu\text{g}/\mu\text{l}$ heptyl stimuli — see Fig. 2). This point can be regarded as a Euclidean vector representation of the stimulus over the interval of interest, produced by a hypothetical array of glomeruli. If the same odor is sampled many times, the points will be distributed in this *glomerular stimulus response space* around a mean response. We will regard this mean response as an average representation of the odor in question and refer to it as a *template*. The variance or standard errors (Fig. 2) associ-

ated with this mean, provides an indication of the variability in the representation of the odor in question. The inner product of the vector representing the response of any single sampling of a particular odor with the average response to that odor can be regarded as an indication of how far that response deviates from its template (a value of 1 indicates exact coincidence), while the inner product of the response vector of a single sampling of an odor with the template of a different odor is an indication of how different the response is from the template of another particular odor (a value of 0 represents the maximum difference since all vectors have positive elements so that their inner products range from 0 to 1).

At each of the four highest concentrations (1, 10, 100, and 600 $\mu\text{g}/\mu\text{l}$) of the hexyl and heptyl suites of stimuli, hypothetical glomeruli were only generated from clusters containing four or more cells. Smaller clusters and isolated cells were discarded in subsequent analyses. Also, for consistency in making comparisons across the full, peak, and post-peak portions of the stimulation interval, the same number of clusters were identified for each of these intervals associated with the same concentration of a particular suite of stimuli (cf. Fig. 2). At the lowest concentration (0.1 $\mu\text{g}/\mu\text{l}$), too few cells responded over some of the stimulation subintervals to perform the analysis presented here. At the highest concentration we only had sufficient cells to obtain two clusters and associated tuning spectra, while at the three remaining concentrations we obtained three (hexyl 10 and heptyl 1) or four (hexyl 1 and 100, heptyl 10 and 100) cell clusters and associated tuning spectra.

4. Linear separation of stimuli

The purpose of the analysis we present here is to obtain some insights into the temporal subinterval of the response spectrum that potentially contains the most useful information for odor discrimination. Our methods are based on the separation of points in a linear response space. Undoubtedly, various non-linear pattern recognition methods associated with different types of neural networks can learn to discriminate the

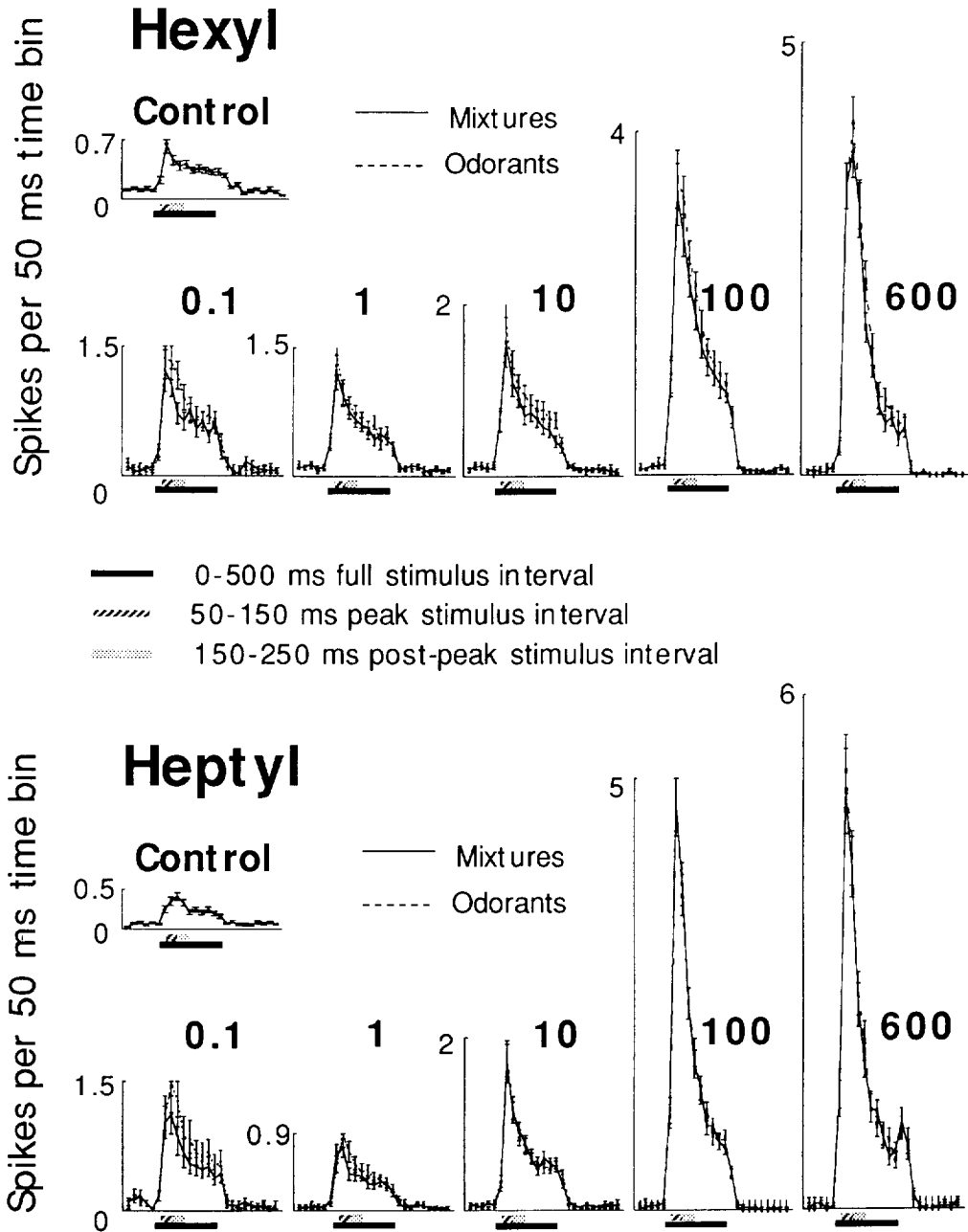


Fig. 1. The average number of spikes per 50 ms time bin and associated standard errors are plotted for groups of receptor cells (19–46 cells in each of the plots, depending on odor concentration 0.1–600 $\mu\text{g}/\mu\text{l}$) from 0.25 s prior to the onset of the 0.5 s stimulus to 0.5 s after the end of the stimulus. The responses to the heptyl (A) and hexyl (B) involve separate populations of cells, although the pure and binary responses involve the same populations of cells. The latter were not combined since we wanted to check for differences that might arise due to inhibitory effects that are known to be associated with the response of sensory cells to binary odors (see text for details).

Heptyl 100 $\mu\text{g}/\mu\text{g}$ clusters

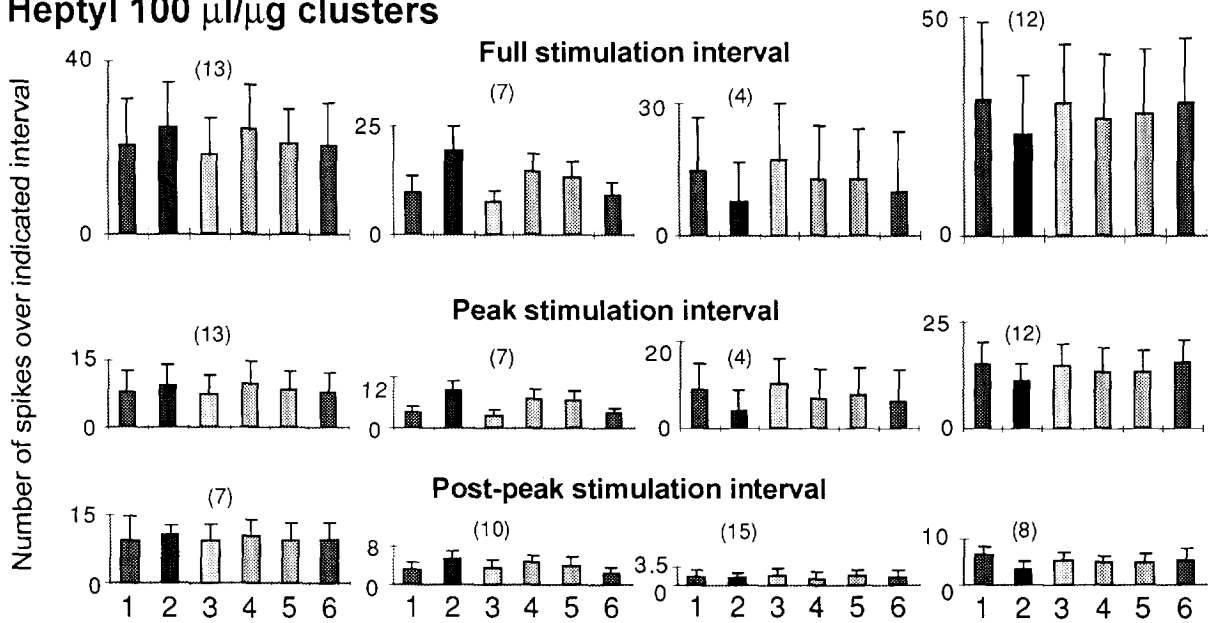


Fig. 2. Each group of six bar diagrams represents the response of a cluster of cells (mean number of spikes over cells in the cluster for the specified full, peak, or post-peak interval, and associated standard error bars) to the following six odors at concentration 100 $\mu\text{g}/\mu\text{l}$: the three odorants 1-heptanol (1), 2-heptanol (2), 2-E-hepten-1-ol (3), and the three 0.5:0.5 binary mixtures 1 and 2 (4), 2 and 3 (5), and 1 and 3 (6). The numbers of cells in each group are in parentheses.

data better than the linear approach we use. For example, it is known that any bounded, non-linear, continuous, monotone, p -dimensional function can be approximated as closely as we want by a three-layer perceptron with sufficiently many hidden units (Funahashi, 1989; Hornick, Stinchcombe, and White, 1990). However, it is the comparative separation of odors provided by the data for the different sub-intervals that is important; since, typically, the better these data are separated in a linear space, the better they will be separated by non-linear networks in general, and olfactory processing systems in particular.

Consider the situation where we take the inner product of a particular template (either a given template or a template selected at random from a set of templates) with the response of an array of hypothetical glomeruli (i.e. the response of each of the m clusters, where $m=4$ for the data depicted in Fig. 2.) to some odor stimulus. Then we could have a rule, for example, that the odor quality of the stimulus in question should be identified as being the same as the odor quality of

the template with which it is compared if and only if the inner product is larger than some pre-selected value δ . To investigate the statistics of this type of identification for the data we had collected, we used Monte Carlo methods (Ripley, 1987) to generate a large number of stimuli that gave response distributions that converge to the same mean and standard errors as the clusters we obtained directly from our data. Specifically, we assumed the data could be fitted by suitably scaled beta distributions (Feller, 1966), with parameters selected to obtain the appropriate means and standard errors. We used these distributions to generate on a computer 100 'typical' responses to each of the three pure odorant stimuli in question. Note that if the variance was relatively large compared with the mean, then a beta distribution is no longer suitable, in which case we used a truncated gamma distribution to generate responses that fitted the given mean and standard error values.

We bootstrapped curves representing the proportion of correct identifications that would be

made if we confined our attention to the pure odorants alone. Specifically, the 100 samples generated for each of the three types of odorant stimuli were used to calculate 300 inner products of each odorant with the template representing a particular odorant quality. The same 100 samples were then used to generate 600 inner products of each stimulus with the two templates of different odorant quality. Note each inner product represents a new random sampling of a stimulus of a given quality. For a selected value δ between 0 and 1 — we refer to δ as a *similarity threshold parameter* — we identified a stimulus as having the same odorant quality as a particular template if and only if the inner product of the stimulus with the template was greater than or equal to δ . We used the proportion of times that an odor was correctly accepted or correctly rejected for each value of δ to obtain the proportion of correct identifications for that value of δ when any of the 300 samplings was randomly compared with any of the three templates.

Since only three odorants are involved, the probability that an odor is misclassified under a purely random process is $1/3$. Therefore, proportions of correct identifications greater than $1/3$ represent improvements obtained for particular values of δ . If δ is selected to be 0, then all stimuli will be identified with the odorant quality of the template and, since all three odorants are equally likely as stimuli, the probability that the identification is correct is $1/3$. On the other hand, if δ is selected to be 1, then all stimuli will be rejected as having the same quality as the template in question. (Note, we assume here that no two stimuli have identical responses to the population means. In reality, some of these may be identical because the precision of any computation is finite.) So for our case of three equally likely odorant stimuli, the probability of rejection being correct in this extreme case is $2/3$.

We plotted the proportion of correct identifications as a function of the similarity threshold δ (Fig. 3) so that comparisons can be made between the performance of the data (total spike counts) from the full, peak, and post-peak response intervals of the olfactory sensory cells to stimulation by one of the three hexyl or heptyl odorants in

question at the indicated concentrations in $\mu\text{g}/\mu\text{l}$. Note, for the sub-intervals and stimuli presented in Fig. 3 (heptyl at $100 \mu\text{g}/\mu\text{l}$), the proportion of correct classifications is maximized by the peak subinterval data at the value 0.71 when $\delta = 0.93$. For $\delta > 0.95$, however the post-peak data produces fewer errors and has a maximum value of 0.70 when $\delta = 0.97$.

5. Results

As described above, we defined the quality of an odor to be a direction in a linear vector space and applied Monte Carlo methods to measure how well the clusters of cells identified for each concentration are able to discriminate between various odor stimuli in terms of how well these stimuli match 'quality template' directions. Examination of the results, pertaining to the peak, post-peak and full interval responses, obtained from two completely independent experiments using different sets of stimuli (Fig. 3) suggest:

1. The proportion of correct identifications decreases with increasing concentration. The reason is that at higher concentrations the activity of all clusters increases with respect to all stimuli and the vectors associated with the stimuli and quality templates become more crowded towards the center (i.e. the 45° line with respect to all axes) of the representation space, thereby degrading the discrimination process.
2. At the higher concentrations — specifically 10, 100, and $600 \mu\text{g}/\mu\text{l}$ — the data obtained from the peak period performed better than the full interval in terms of the proportion of correct identifications made, while the data from the post-peak interval performed the worst. This hierarchy of performance cannot be attributed to increasing spike activity, as discussed under the previous point, because the relationship is not congruent: the full interval contains more spikes than the peak interval (not the other way around), while the post-peak interval contains the least.
3. At a concentration of $1 \mu\text{g}/\mu\text{l}$, the data from the post-peak interval performed best, al-

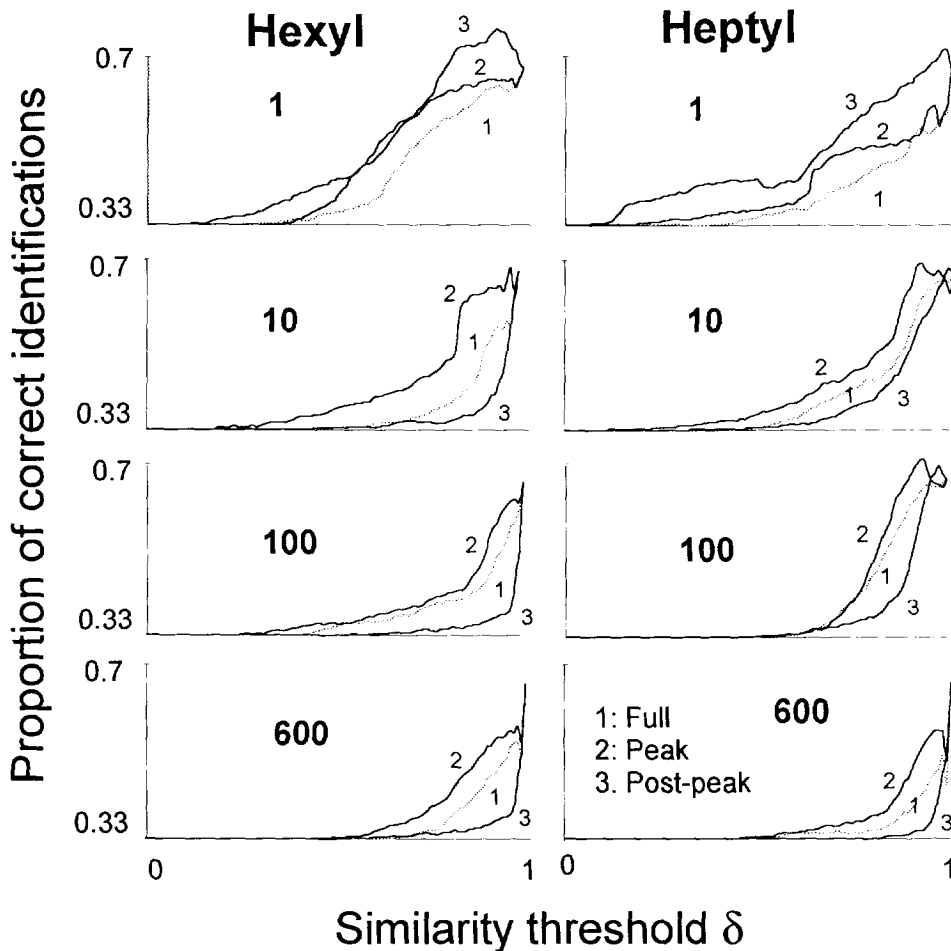


Fig. 3. The proportion of correct identifications are plotted as a function of the similarity threshold δ for the stimulus data, as labeled in terms of concentration and odor suite. The response intervals concerned (full, peak, and post-peak) are labeled on each of the individual graphs.

though in the hexyl case only for values of the threshold parameter δ greater than about 0.8. The data obtained from the full interval performed worst in all three cases, possibly for the reasons discussed under point 1 (i.e. because more spikes are involved).

4. If it is more important to minimize type II errors (the probability of incorrectly accepting a stimulus) than type I errors (the probability of incorrectly rejecting a stimulus), as would be the case if an individual needs to avoid a highly poisonous substance, then a value of δ close to 1 is required. In this case, the post-peak information becomes relatively

more important, as indicated by the dominance of the post-peak 'correct identification' curves for the higher values of δ in the hexyl 1, and heptyl 1, 10 and 100 cases.

6. Conclusion

Since the processing of olfactory signals is dynamic (Li and Hopfield, 1989; Getz, 1991; Hopfield, 1991; Linster et al., 1993; Rospars and Fort, 1994; Linster and Masson, 1996), it is misleading to think of the quality of any odor as being represented by a static 'across fiber' pattern in the

relay neurons of the antenno-glomerular tracts. Rather, as illustrated in Fig. 1, the responses of olfactory sensory neurons are dynamic even for constant stimuli, and the responses become more variable if the stimuli are variable in space and time. Further, the pattern of responses are known to change with concentration (Smith and Getz, 1994). Thus it is important to our understanding of olfaction in insects to have some sense of the temporal dimensions of the process.

From proboscis extension conditioning experiments with honey bees, it appears that discrimination of odors takes no longer than a few hundred milliseconds (Smith and Menzel, 1989). If the same time frame applies to cockroaches, then our results are consistent with the peak 50–150 ms response period being the interval that needs to contain the most information on the quality of the stimulus. At lower concentrations, a longer period of time may be required before sufficient spikes are generated to overcome any background noise and the quality of a particular odor stimulus can be perceived. This is consistent with the post-peak interval of 150–250 ms containing more useful information at lower than at higher concentrations (Fig. 3).

If olfactory patterns vary greatly with the concentration of stimuli of given quality, then we need to resolve how the quality of a stimulus can be perceived over a range of concentrations. The resolution may be related to the fact that spatial and temporal variability of stimuli (Murlis et al., 1992) naturally lead to variation in concentration over a short interval of time. In this case, a collection of input patterns, representing the response to the same stimulus but at different concentrations, become associated with one another because they occur together over short enough periods of time. We should expect that some of the deeper problems in insect olfaction can only be understood in terms of the dynamics of neural nets. Network models of olfactory processing do exist (e.g. Li and Hopfield, 1989; Getz, 1991; Hopfield, 1991). In insects, these models typically are designed to replicate observed firing patterns in antennal lobe neurons (Linster et al., 1993; Linster and Masson, 1996).

The data we present here provide some pointers to the questions we should be asking of antennal lobe function when developing neural network models of olfactory processing in insects. In particular, we need to be aware of the time constants associated with the sampling of stimuli (Theunissen and Miller, 1995) and how these time constants may change with respect to concentration, position in space, and over time. We also need to account for the context of the stimulus in terms of whether it is more important to reduce errors associated with accepting or with rejecting a preconceived notion that a stimulus has a particular odor quality.

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