to regulate different ion channels. Since morphological and physiological comparisons of the vertebrate and insect olfactory systems have revealed remarkable similarities at the periphery as well as in the brain (Boeckh et al. 1965; Lancet 1986; Anholt 1987; Breer 1997; Kaissling 1987; Homberg et al. 1989; Shepherd and Greer 1990; Hildebrand and Shepherd 1997) it might be possible that they also share olfactory transduction mechanisms. How similar the transduction mechanisms in the different phyla actually are, however, cannot be judged yet and will be the focus of future studies. The present knowledge only allows speculation about the interactions of the second messenger systems involved, and how they regulate the ion channels present in the different compartments of the ORNs.

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1) Note added in proof:
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Vosshall et al. Cell 96:725–736 (1999)
Clyne et al. Neuron 22:327–338 (1999)

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CHAPTER 3

Function of Peripheral Olfactory Organs

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Contents

1 Introduction

The function of the olfactory receptor organs of insects is to send the central nervous system (CNS), on a split-second basis, a rendering of the relative abundance of single odorants that together comprise odour blends. The organs' olfactory receptor neurons (ORNs) generate action potentials (spikes) that are transmitted to the antennal lobe (AL) (Chaps. 4 and 5), which then sends the processed signal on to higher integrative centers of the brain (Chap. 9). When an ORN is hit by molecules of an active stimulus, the molecules are thought to interact with receptor proteins situated in the dendritic membrane (Chap. 2). These interactions form a receptor potential that travels down the

dendrite to a site where action potentials are triggered when the receptor potential reaches a threshold level. The action potentials travel down the length of the ORN axon into the AL (Chap. 5). The action potentials of particular ORNs have characteristic sizes and waveforms (Kaissling 1974) that often differ greatly enough among ORNs that researchers can discriminate them and assess their activities separately. Spike sizes may be related to the size of the ORNs generating them (Hansson et al. 1994b), but the size of the spike is not known to carry any extra information to the AL. It is the frequency of spikes rather than their size that is important for generating behavioural responses.

Information about the absolute concentration of odour also may be important to the insect, but instances in which this has been shown unequivocally are rare. We know from the preponderance of behavioural studies that what is most important to insects is the quality of the odour and changes in its concentration. The peripheral ORNs allow the brain to assess odour quality by being tuned to different types of molecules, and due to their differential responses to these molecules, to provide patterns of activity, inactivity, and even hypoactivity (Boeckh 1967, Dethier 1972) that characterise odour blends containing different proportions of these molecules. Dethier (1972) pointed out the importance of these unique patterns, called across-fiber patterns, to larval lepidopteran olfactory behaviour, with fibers referring to the axons of the ORNs reporting to the brain (Fig. 1). In addition, he illustrated how the hypoactivity of some of the ORNs generating spikes at below their baseline firing rates to some odour consituents could provide the brain with even richer blend-discrimination possibilities (Fig. 1).

Sass (1978) and Selzer (1981) showed how the patterns of activity from the known ORNs on American cockroach [*Periplaneta americana* (L.)] antennae differed in response to synthetic single odorants (Fig. 2) and to known natural blends of odorants such as those emanating from orange, banana, meat, and cheese (Fig. 3). These patterns can only be assessed by higher-level interneurons, beginning with AL interneurons. But it is the antennal neurons' differential responses that make the pattern and the rendering of the odour quality possible.

Information regarding the presence or absence of the odour is provided first by the sensitivity of the individual ORNs, and then by their collective sensitivity. Collective sensitivity is produced by having a number of ORNs from across the antenna tuned to a particular odour component converge upon and synapse with a limited number of interneurons. Collective sensitivity depends on the ratio of the numbers of similarly tuned ORNs to the number of interneurons on which they converge (Boeckh and Boeckh 1979; Boeckh and Ernst 1983; Chap. 5), which could conceivably be heightened by each ORN having greater numbers of synapses on any interneuron. In insects, the convergence for, and therefore sensitivity to, pheromone components is very high

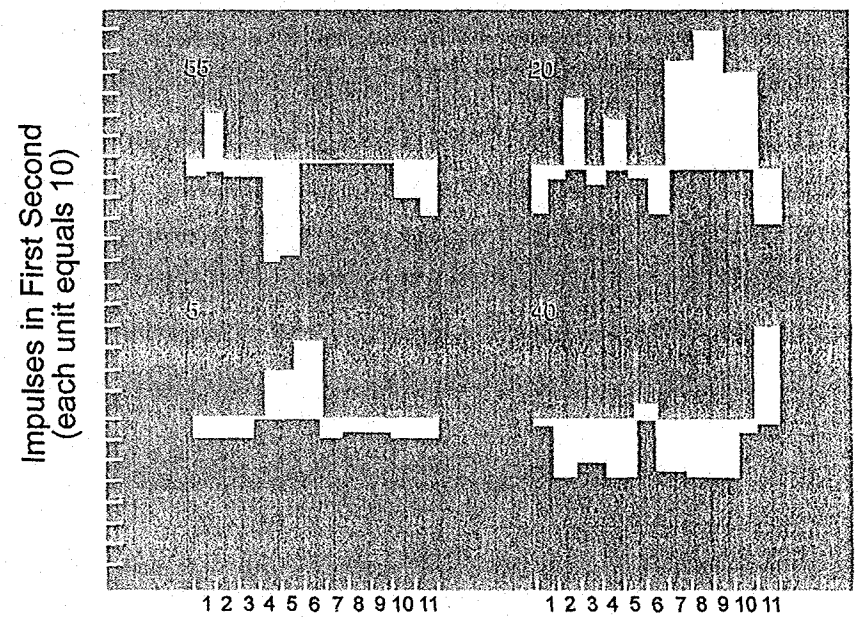


Fig. 1. Patterns of activity across four different olfactory neurons on *M. sexta* larval antennae in response to 11 different odorants. It can be seen that any single odour creates a fairly distinct ratio of firing from the four neurons. For instance, odour number 11 is the only odour that creates an increase in firing from neuron number 40 (*lower right*) and decreases from neurons 55, 20, and 5. Odour numbers 8 and 9 create a similar ratio of firing from the four neurons (increase in firing from neuron number 20 (*upper right*), slight decreases from 5 and 40, and no change from neuron 55), and these two odours possibly would not be discriminated by the animal. In contrast, odour 10, while creating this similar pattern in neurons 20 and 5, stimulates neuron number 55 to decrease firing and neuron number 40 to change very little from its basal rate, and this odorant possibly would be discriminated from odours 8 and 9. X-axis displays number of nerve impulses elicited during the first stimulation of odour stimulation. From Dethier (1972)

compared with that to food odorants, as exemplified by the American cockroach (Boeckh and Ernst 1983; Chap. 4). Because pheromone blends are usually very simple, often comprising just two components (as in the American cockroach), pheromone-component-sensitive neurons can be multiplied tens of thousands of times and devoted only to amplification of this simple blend. In contrast, discrimination of food odours that are complex, and contain thousands of potential odorants from scores of odorant classes, requires that ORNs be partitioned into a greater variety of classes, each with a tuning spectrum that is broader for reporting this wider variety of odorants (Hildebrand and Shepherd 1997). The potential to amplify, and be as sensitive to, food odours, while meeting the challenge of discriminating them

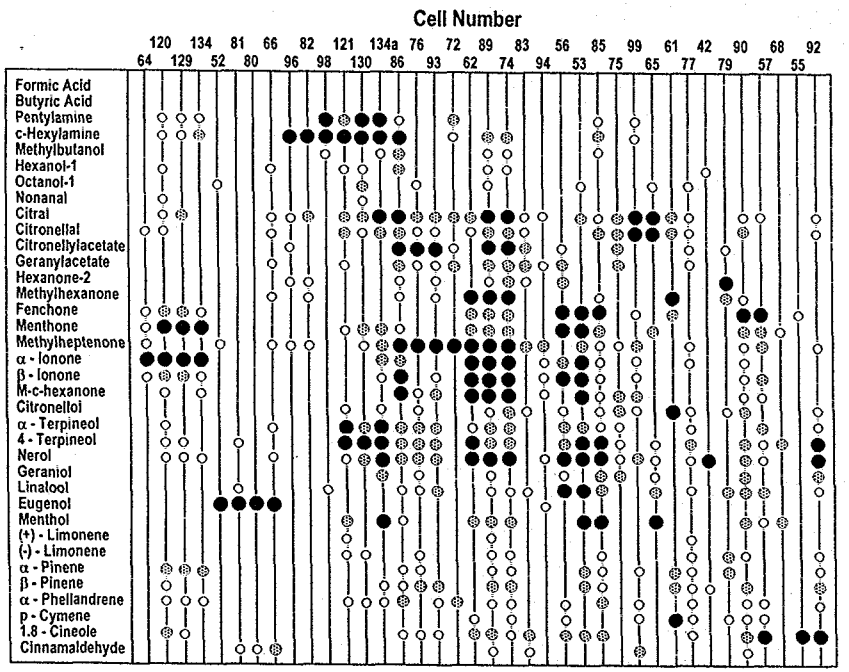


Fig. 2. Patterns of activity from ORNs on the antenna of *Periplaneta americana* that are sensitive to terpenes and alcohols. Responses are standardised to the neurons' responses to the most effective stimulus. It can be seen that patterns of response to even single odorants such as these create different patterns across this ensemble of neurons, many of which could be discriminated by interneurons in the antennal lobe and higher. Small (*open*) dots indicate reactions of neurons up to 25% of their maximal reaction, mid-sized (*stippled*) dots indicate responses up to 50% of their maximal reaction, and large dots (*black*) indicate >50% of maximal response. From Seltzer (1981)

would thus not seem to be as great as for the simpler pheromone odours. Perhaps that is why most insect pheromone blends are so simple; long-distance sensitivity can be optimised while keeping discrimination at a maximum by using as few components as possible.

2 Behavioural Background

Absolute concentration levels do affect insect behaviour, as evidenced by differences in the behaviour of flying insects in response to different emission rates of odour at a fixed distance from the source (Cardé and Hagaman 1979; Baker and Kuenen 1982; Kuenen and Baker 1982). However, such responses

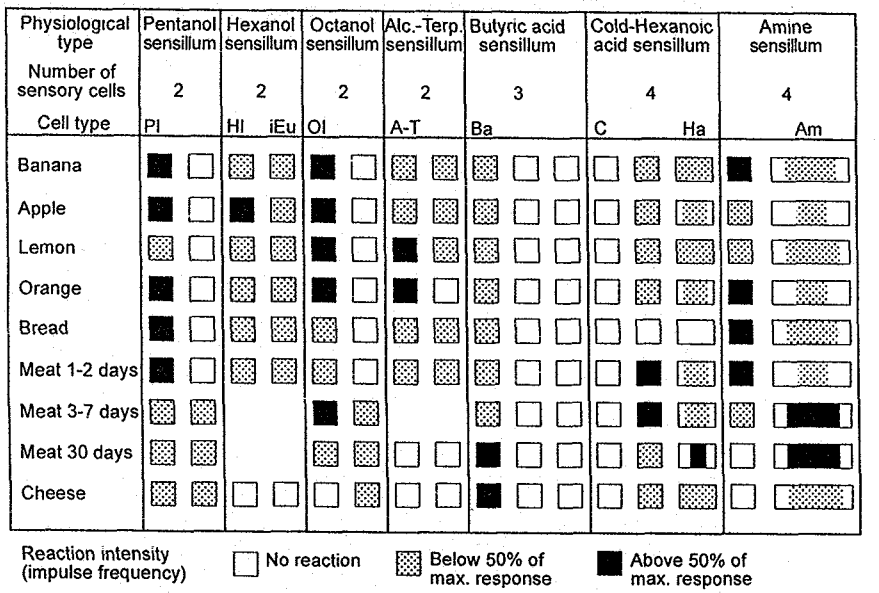


Fig. 3. Patterns of activity from ORNs on the antenna of *P. americana* related to odour quality discrimination of food items. The response of this ensemble of differently tuned neurons creates a unique ratio of firing in response to each food item that can potentially be integrated and responded to uniquely by higher-order interneurons in the antennal lobe. From Sass (1978)

to concentration levels should not lead to the (teleological) conclusion that insects use concentration to assess their distance from the source. There are several reasons why distance-to-source measurements by insects based on concentration may be relatively unimportant. First, adaptation can quickly alter the ability of ORNs to give such readings. Second, odour plumes even far downwind of an odour source are known to contain filaments of odour that are nearly as concentrated as filaments close to the source (Murlis 1986) and so distance-to-the-source cannot be easily (if at all) unequivocally discriminated by the insect by using absolute concentration readings. Although it is true that these virtually undiluted odour bursts are rarer farther downwind than closer to the source, it is unclear how the insect would be able to discern infrequent far-downwind encounters from those occurring closer to the source caused by the meandering of either the plume over the insect or the insect over the plume. Third, although in an undisturbed plume the rise and fall slopes of filaments are shallower farther downwind (Murlis 1986, 1997),

such diffused filaments could easily occur close to the source as well, having been generated by turbulence from surrounding vegetation. Finally, measurements have shown that the frequency of filament encounters will not differ with distance (Murlis 1986, 1997), and the frequency is dependent on the animal's own airspeed, especially at lower windspeeds (Baker and Vickers 1994).

Most importantly, there is no behavioural evidence that orientation to an odour source requires, or even involves, distance-to-source knowledge. All of the mechanisms shown thus far to result in successful odour-source location fail to include such distance assessments (Vickers and Baker 1994, Mafra-Neto and Cardé 1994). Even close to an odour source – and that includes along insect trails such as those produced by termites, ants, and gregarious caterpillars in which the trail follower is never more than a few millimeters from the source – there is no evidence that anything other than the relative difference in concentration, either in time or space (between two antennae), is used for steering. For instance, Rust et al. (1976) found that American cockroaches can steer toward the source of sex pheromone when within 70 cm of the source in completely still air when they have two intact antennae. With only one antenna, they do not turn correctly toward the source more than expected by chance alone. Argentine ants, *Iridomyrmex humilis* (Mayr), can orient using olfaction alone along trails of their trail pheromone when these are inverted above the ants so that they are walking in an odour corridor without being able to touch the deposited trail (Van Vorhis Key and Baker 1981).

The functions of sensory neurons important to behavioural responses to odour can only be grouped by categorising the known behavioural responses themselves. Insects that walk toward a source of odour appear to use systems that are analogous to those used by flying insects, except that optomotor responses are not necessary (Kaissling 1997). For insects that locate odour sources by flying, the orientation mechanisms used seem to be similar, regardless of whether they are responding to sex pheromone (Kaissling 1997), host-plant odour (Zanen and Cardé 1996), or host-animal odour (Gibson et al. 1991, Cossé and Baker 1996). The overlap between host odour and pheromone response systems is illustrated most graphically by the antennal transplant studies of Schneiderman and Hildebrand (1985), in which female *Manduca sexta* (L.) possessing male antennae flew upwind to pheromone in a manner similar to males, and in a fashion similar to when the females flew upwind to host odour by using their own intact antennae. Loss of odour causes a quick change from upwind progress to a more crosswind flight track (e.g., tsetse flies [Gibson and Brady 1988, Gibson et al. 1991]), often coupled with more or less temporally regular reversals (e.g., casting flight in moths [Kennedy, 1983, 1986; Haynes and Baker 1989]) or *Drosophila* (David 1982). When odour is lost by a walking insect such as a cockroach or moth, it will

in some cases oscillate left and right quickly (Kanzaki et al. 1992, Kanzaki 1996) before circling or looping (Kramer 1986; Tobin and Bell 1986; Kanzaki 1996).

Responses to both contact with and loss of sex pheromone filaments by male moths can be as fast as 0.15 s [*Grapholita molesta* (Busck), Baker and Haynes 1987], but usually are between 0.3–0.6 s [*Heliothis virescens* (F.), *Helicoverpa zea* (Boddie), and *Antheraea polyphemus* (Cramer), respectively; Vickers and Baker 1996, 1997; C. Quero, H. Fadamiro and T. C. Baker unpubl. data, Baker and Vogt 1988]. The latency of the casting flight response to loss of host-plant odour is similar to that of the latency for pheromone loss, 0.7 s (Haynes and Baker 1989). The latency of a change from crosswind flight to upwind flight in response to contact with host-animal odour also appears to be in the order of 0.3 s (Colvin et al. 1989).

The rapid response to an odour filament, or strand, is important because the strand has been shed from the odour source and in higher-velocity airflow, continues to travel in a more or less straight line away from the source even though large swings in wind direction seem to make the entire plume meander left and right. The responding insect can move in a straight line directly toward the source if it flies upwind when it smells the odour. The plume's large meanderings, however, create large areas of clean air into which the upwind-advancing insect can fly (David et al. 1982; Elkinton and Cardé 1987). Thus, it is just as important for the insect to respond quickly to clean air as it is for it to respond to an odour filament, because to continue upwind following odour loss for any length of time is to plunge too far into the clean wind, which will lead away from the lost odour plume and off-line from the odour source (Baker 1990, Kaissling 1990).

Thus, based on the behavioural evidence, the ORNs of flying insects must be able to report changes in odour concentration rapidly to the brain such that it can quickly discriminate odour quality and allow the insect to react quickly to both increases and decreases in concentration. Second, the long-lasting responses such as casting by flying insects or looping by walking insects in clean air following odour loss must be driven by the olfactory system during long clean-air gaps. These types of tonic responses would serve to optimise the insect's chances of recontacting the lost odour strands in a meandering plume. Such long-term response of interneurons in the brain has been shown in at least two species (Christensen 1989; Kanzaki et al. 1989; Christensen et al. 1991, 1995a; Chap. 9), interestingly only when the correct blend is used for stimulation, but there are only a few known examples in which pheromone ORNs continue to respond tonically after stimulation has ceased, long enough to account for the sustenance of casting flight in a pocket of clean air. It is not known whether prolonged movement in clean air such as casting flight (or looping during walking) is driven in any species by the tonic activity of some portion of the population of ORNs or only by the tonic

activity of CNS interneurons. Recently, evidence has accumulated that another function of the olfactory organs is to report sufficiently fine-grained information that the insect can discriminate a plume in which every filament contains a complete blend of odour from one in which the blend is partitioned into separate filaments (Baker et al. 1998).

3 Properties of Peripheral Olfactory Receptor Neurons

To perform their functions of providing the brain with an accurate rendering of the quality of the odour, its presence or absence, its split-second changes in concentration, and the spatial or temporal noncoincidence of odour strands, ORNs must have certain response properties built into them. The receptor properties that we will focus on in this Chap. are as follows: (1) selectivity, or how narrowly an ORN is tuned to one odorant; (2) sensitivity (i.e., an ORN's reaction to concentration, including changes in concentration); (3) co-compartmentalisation of neurons within sensilla, related to spatial and temporal odour resolution; and (4) relative rates of adaptation and disadaptation.

3.1 Selectivity

One property common to all insect ORNs is the selectivity that they exhibit in responding most intensely and with the lowest thresholds to specific kinds of molecules. The molecule or type of molecule to which an ORN displays the lowest threshold is the one to which the ORN is said to be tuned. The tuning may be optimised, for example, for a particular molecular shape, length, electron distribution, position of double bonds, or functional group. Selectivity appears to be imparted at all stages of the molecule's migration from the cuticle through the perireceptor environment to the target neuron (Chap. 2). Resident neurons in the receptor organs of nearly every species display specificity of one degree or another to a relatively narrow range of molecules, be they host-odour components or pheromone components (Boeckh and Ernst 1983, Hansson 1995).

An example of how host-odour-sensitive neurons are tuned comes from the American cockroach (Fig. 4; Sass 1978). The three ORN types from which recordings were made displayed the lowest thresholds to three different types of aliphatic alcohols, either according to the molecules' chain lengths or whether or not they contained a methyl branch. The tuning of the first neuronal type to 3-methyl butanol is not absolute. Similar molecules can cause this neuron to fire with the same intensity as 3-methyl butanol, but it takes 10, 100, or 1000 times the concentration to do so.

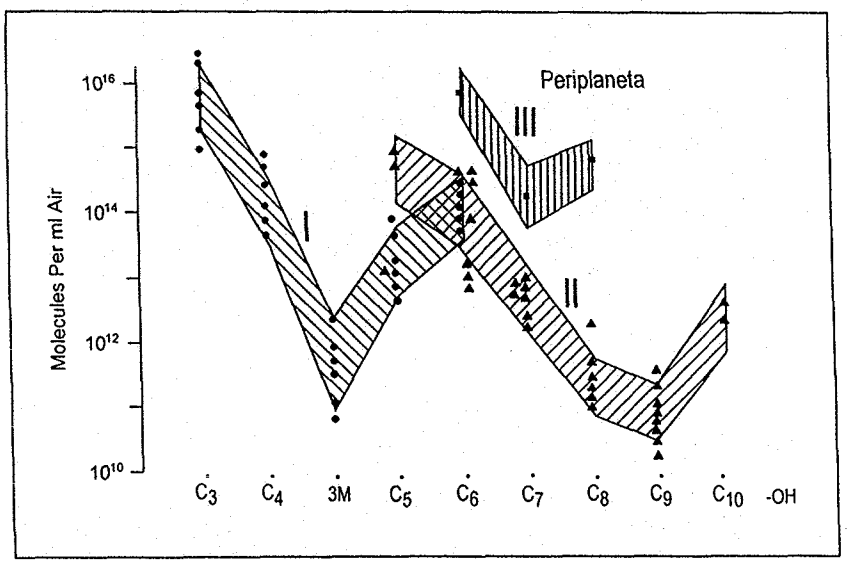


Fig. 4. Tuning of three ORN types on the antennae of *P. americana* that are sensitive to aliphatic alcohols. It can be seen that neuron type I is more responsive to shorter- rather than longer-chain alcohols, with peak tuning (lowest threshold) for 3-methylbutanol (3M). In contrast, neuron type II is more sensitive to longer-chain alcohols, with peak tuning for n-1-nonanol (C9). Neuron type III, although less sensitive in general to these alcohols, responds with the lowest threshold to n-1-heptanol (C7). The pattern of firing of these three types of neurons alone, without the inputs from any of the other types of ORNs on the antenna, would probably allow for higher-order interneurons to discriminate among any of the alcohols in this series. From Boeckh and Ernst (1983). Shaded areas in the three curves represent concentration ranges of each compound that will cause the ORN to fine at 50% of its peak frequency

Even though this ORN is tuned to 3-methyl butanol, its imperfect affinity for this molecule means that unambiguous discrimination for the concentration of 3-methyl butanol in the environment, or even just its presence, cannot be achieved by the "3-methyl butanol ORN" alone. One can easily see (Fig. 4) that if this type of neuron were firing with 50% of its capacity (shaded area of curve at left), the animal could not discriminate whether the firing was due to low concentratons of 3-methyl butanol or to 100 times higher concentrations of n-butanol, n-pentanol, or hexanol. Thus, it could not discriminate 3-methyl butanol from other odorants in the environment with the 3-methyl butanol ORN alone.

The peripheral olfactory system can optimally provide the CNS with information sufficient for odour quality discrimination only when it is built with at least one other neuronal type with a tuning curve overlapping with the first

type of neuron, as represented by the neuronal type to the right in Fig. 4, which is tuned optimally to n-nonanol. A 50% activity level from this neuron, coupled with the same level of activity from the first neuronal type would immediately be registered in the CNS as coming from the presence of either n-pentanol or n-hexanol, and there would be no question that the activity from neuron type 1 is not due to 3-methyl butanol. In contrast, if the second neuronal type were silent while the first type was firing, the CNS interneurons would be able to discriminate that the molecule in question was either a low concentration of 3-methyl butanol, a high concentration of n-butanol, or a very high concentration of n-propanol. If there were a third neuronal type tuned to n-propanol, this uncertainty would be eliminated as well, and discrimination would be enhanced yet again.

Thus, the narrow selectivity of each of the many types of peripheral olfactory neurons that are typically present in insect systems does not in itself allow for odour quality discrimination. The selectivity does, however, provide the necessary raw signal about that kind of molecule, or about higher concentrations of molecules that are very similar. Likewise, a particular molecular concentration cannot be discriminated without discrimination of the odour quality. The resolution of quantity and quality, even for an odour comprised of a single type of molecule, can only come from an across-fiber comparison, or pattern, made by the CNS, of the activities of ORN types having overlapping response spectra (Dethier 1972; Boeckh and Ernst 1983; Chap. 5).

There are other excellent examples of the activity of ORNs contributing to odour-quality discrimination by means of their selective tuning and differential responses to particular odorant molecules. Work by den Otter and colleagues has identified ORNs on tsetse fly antennae (*Glossina morsitans morsitans*) that are differentially tuned to several odourant molecules important to host finding. They found ORNs exclusively tuned to 1-octen-3-ol, to acetone, to dichloromethane, to 3-methyl phenol, and to CO_2 (den Otter and van der Goes van Naters 1992). Differences in the patterns of firing of these ORNs would certainly allow the flies to discern differences in blends deficient in one or more of these odourants. Further work with a series of phenol analogs disclosed still finer tuning of the phenol-sensitive ORNs (den Otter and van der Goes van Naters 1993). Some of them responded most strongly to 4-methyl phenol and some were strongly inhibited by 3-methyl phenol but not by other phenols, and some responded with the same degree of excitation to every phenol presented. The authors pointed out that these different responses would present different across-fiber patterns to the CNS and allow the flies to discriminate for nearly every phenol (den Otter and van der Goes van Naters 1993).

Work by several groups with aphids has revealed ORN systems whose differential specificities would give the CNS a high degree of odour-quality specificity (Bromley and Anderson 1982; Nottingham et al. 1991; Hardie et al. 1994b). Bromley and Anderson (1982) found that placoid sensilla in the primary rhinaria of the antennae of the lettuce aphid, *Nasonovia ribis-nigri*, contained ORNs with differential tuning to a wide variety of odorants that might be involved in host plant location. They concluded that differences in the ORN responses of the distal primary rhinaria compared with the proximal primary rhinaria to these green leaf volatiles could allow discrimination of different plant odours. In addition to more subtle differences in the responses to green leaf volatiles and aldehydes, there were great and consistent differences in the ORN responses to terpenes, with the ORNs from the distal rhinaria generally being inhibited by terpenes and those from the proximal rhinaria being excited (Bromley and Anderson 1982). Recordings from the proximal primary rhinaria of the black bean aphid, *Aphis fabae* (Scopoli) revealed a class of ORNs tuned to isothiocyanates, which are not emitted by their host plants but are emitted from brassicaceous nonhost plants (Nottingham et al. 1991). It was hypothesised that these ORNs would aid in discrimination between host and nonhost plants, all of which emit other common volatiles such as green leaf volatiles. Finally, it appears that ORNs in the secondary rhinaria of male *A. fabae* are specialised for responding to female-emitted sex pheromone, and would aid in discriminating sex pheromone plumes from plant-odour plumes for males responding directly to the female emission (Hardie et al. 1994).

Mosquitoes have been shown to be capable of discriminating odours of different quality. Studies have implicated specifically tuned ORNs as contributing to this discriminatory ability. Apparent discrimination for plant odours for plant feeding by *Culex pipiens* L. is facilitated by ORNs tuned to terpenes, which are not part of animal-host emissions (Bowen 1992). The malaria mosquito, *Anopheles gambiae*, is a human host-specific species that does not need carbon dioxide (CO_2) as a constituent of the host odour emission. Adult females can locate human hosts solely by means of a blend of skin-emitted volatiles, especially from the foot region of the body (De Jong and Knols 1995a,b) and this attraction can be approximated by the odour emissions from Limburger cheese (De Jong and Knols 1995b). Using electroantennogram (EAG) recordings, Knols et al. (1997) found that there is a class of ORNs on *A. gambiae* antennae tuned to the short-chain fatty acids that are predominant in Limburger cheese as well as foot-odour emissions, with optimal responses appearing to occur to acids in the range from hexanoic to octanoic. That an anthropophilic species of mosquito such as *A. gambiae* should possess ORNs specifically tuned to short-chain fatty acids that are unique to the emanations from human skin compared with other animals indicates a role for these ORNs in host-odour discrimination (Knols et al. 1997). Species that are more opportunistic feeders on a wider variety of animal hosts, including humans, could rely on the general animal emission CO_2 as a stimulus in

combination with other general animal volatiles. The ORNs on the maxillary palps that have been shown to be tuned specifically to CO_2 in a large number of mosquito species (Grant et al. 1995) would aid in such animal-host blend discrimination and in host location.

Recent evidence has established that the selective tuning of the peripheral olfactory organs is accomplished by both the perireceptor environment housing the neuronal dendrites, as well as the receptor sites on the dendrites themselves (Steinbrecht et al. 1994; Prestwich and Du 1997; Chap. 2). The extent of this specificity is, however, germane to our discussion, and some of the most detailed studies examining this aspect of the functioning of ORNs have been performed by Liljefors et al. (1984, 1985, 1987; also Bengtsson et al. 1990). This research team made various well-chosen modifications of the structure of (*Z*)-5-decenyl acetate (Z5-10:Ac), one of the three pheromone components of *Agrotis segetum* Schiff., and then tested their activity on the ORN that is tuned to this component. Liljefors et al. (1984, 1985, 1987) accounted for any differences in the volatility of the Z5-10:Ac analogues caused by differences in chain length or functional groups, and then measured the firing rate of the neuron in response to the compound of interest. Several relationships between the structure of the molecule and the neuronal response became clear. First, the affinity of the ORN was related to the shape of the molecule, including its length and bulkiness. Second, changes in the position of the double bond significantly affected the firing frequency of the ORN, and thus both the electron distribution associated with the double bond's distance from the acetate group and the length of the alkyl chain were responsible for some of the specificity. More evidence that electron distribution is related to the molecule's interaction with the ORN came from the replacement of the hydrogens with fluorines on the carboxyl group forming the acetate end of the molecule (Liljefors et al. 1984). Whereas molecular models showed that the bulkiness, shape, and volatility was nearly completely unaffected by this replacement, the Van der Waals forces were nearly 180° reversed from the natural ligand. This reversal accounted for the nearly complete loss of ORN activity.

The Liljefors et al. (1984, 1985, 1987) and Bengtsson et al. (1990) studies gave other insights into ligand-related ORN activity in insect olfaction. One important additional insight came from their use of conformational energy models (Liljefors et al. 1985). They pointed out that odourants such as pheromone-component molecules are not rigid, but rather have various abilities to bend and rotate depending on their structure, when in solution and away from receptor or binding-protein pockets. When they interact with other molecules with which they can bind, they bend to conform to the shape and the charges within the binding pocket and will thereby have a fixed geometry. The Liljefors team found that when they calculated the energy needed to bend each of the various pheromone component analogues to conform to the

geometry of the most active ligand in its fixed state, this conformational energy was inversely proportional to the frequency of firing evoked in the ORN (Liljefors et al. 1985). This meant that the lower the amount of the energy needed to force the candidate odorant molecule to fit into the pocket, the more it was an effective stimulus to the receptor, and vice versa. This would explain the peculiar and surprising property of extra-long chain-elongated analogues to be more effective stimuli than either slightly chain-elongated or chain-shortened analogues. It takes less energy to fold a very long-chain analogue entirely back on itself and to fit it into the pocket than it does to bend a slightly-too-long analogue to fit the pocket (Liljefors et al. 1985).

These concepts are important for understanding ORN specificity, whether this specificity is imparted at the binding-protein level between the odorant ligand and the binding protein, or at the membrane-bound receptor site level with the odorant itself acting as the ligand or the odorant-binding protein complex acting as the ligand (Chap. 2). This type of research shows that the ability of molecules to conform to the shape of a protein and bind to it depends on many factors, and that molecules other than the one the receptor has been designed for can and do fit in and interact with amino acid residues in the pocket and activitate the neuron (Prestwich and Du 1997).

Usually, an analogue of the natural odorant is a less effective stimulus, and a higher concentration is needed to evoke the same firing frequency from a neuron. For example, Davis (1988) presented various analogues of lactic acid to the lactic-acid-specific ORN in *A. aegypti* antennae (Davis 1988). None of the more than 20 analogues tested was as effective at exciting this ORN as lactic acid itself, although several analogues came close. From these results Davis (1988) deduced some of the molecular properties essential for optimal activity and several that are detrimental to activity. However, when an ORN has the same thresholds for two different molecules, interesting behavioural effects can occur that have evolutionary implications. In the ermine moth, *Yponomeuta rorellus* L., the ORN for tetradecyl acetate (14:Ac), the major and only known pheromone component for this species, responds equally well to (*E*)-11-tetradecenyl acetate (E11-14:Ac) and (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), the components comprising the essential blend of several sympatric species of *Yponomeuta* (Löfstedt et al. 1990). *Y. rorellus* is the only species in the Lepidoptera known to use a saturated acetate as its pheromone, and it appears to have achieved this ability by developing a much less specifically tuned ORN than the other species. This ORN seems to have retained an ability to respond to the unsaturated pheromone components used by other species. The firing of this large-spiking neuron in response to either Z11- or E11-14:Ac is not correlated with *Y. rorellus* males being attracted erroneously to females of the wrong species. Rather, adding these unsaturated compounds to a 14:Ac pheromone source eliminates the attraction of *Y. rorellus* males (Löfstedt et al. 1990). There is an ORN present that exhibits medium-sized spikes in the

same receptor hairs that house the large-spiking 14:Ac-sensitive neuron, and it responds to E11- and Z11-14:Ac, but not to 14:Ac. This neuron also fires in response to another molecule known to be antagonistic to attraction, (Z)-11-hexadecenyl acetate (Z11-16:Ac) (Löfstedt et al. 1990).

There are many examples of ORNs that are able to be activated by higher doses of analogues than the natural ligand, but there are very few in which an analogue is as effective as the natural ligand. There are fewer still in which the active analogues are naturally existing odorants, as in the *Y. rorellus* system. *H. zea*'s olfactory system offers another example in which the ORN for both the minor pheromone component as well as the ORN for behavioural antagonists are both tuned equally well to naturally occurring compounds emitted by sympatric females in the heliothine subfamily (see below).

3.2 Sensitivity

Another property of ORNs is their sensitivity to different concentrations of the odour stimulus, either in the absolute sense or in the relative sense (i.e., to changes in concentration). Again, absolute concentration (quantity) of a particular odour cannot be discriminated without its identity (quality) also being discriminated. Changes in overall molecular concentration can be discriminated by single types of ORNs as long as the odour composition also does not change, but if it does, the quality of the odour also must be discriminated before the change in concentration can be measured. Such quality discrimination would require that the variously tuned types of ORNs increase or decrease their firing rates in concert with each other in response to changes in the flux of the particular odorant to which they are tuned. Thus, if the flux of a particular odour blend changes, the relative ratios of firing of the different ORNs will remain consistent and will accurately report that blend ratio to the brain (Fig. 5).

It has been suggested that ORNs, unlike gustatory RNs, are not concentration detectors. Rather, they may more rightfully be considered as flux detectors that can report changes in molecular abundance through time (Kaissling 1998a). This generalisation appears to be true for ORNs tuned to lepidopteran sex pheromone components, and as described earlier makes sense with regard to the behavioural reactions required of moths for locating a sex pheromone source. However, there are examples of some ORNs that behave as both absolute concentration detectors and as flux detectors. Grant et al. (1995) described ORNs housed in sensilla on the maxillary palps of a variety of mosquito species that are tuned to CO_2. ORNs tuned to CO_2 also have been found on the labial palps of moths (Bogner et al. 1986, Bogner 1990) as well as on the antennae of tsetse flies (Bogner 1992, Den Otter and van der Goes van Naters 1992).

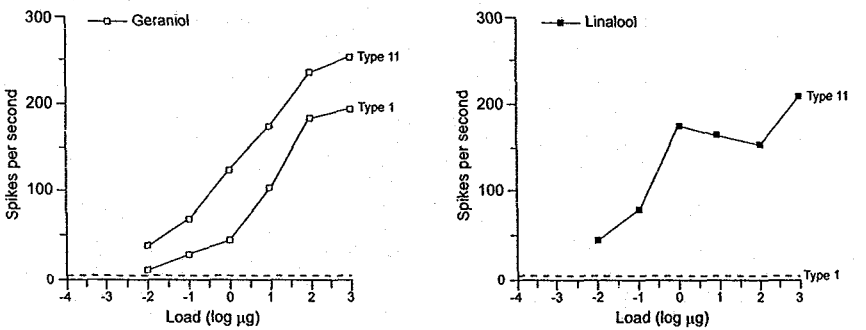


Fig. 5. Responses of two types of ORNs from the antennae of *S. littoralis* to a range of dosages of geraniol (*open squares*). Increasing dosages stimulate proportionally similar increases in firing frequency from both types of neuron. Ratios of firing integrated in the antennal lobe would thus be similar across a wide range of odour plume filament concentrations and likely be discriminable as geraniol across these dosage ranges, even considering only these two cell types. Linalool also stimulates cell type 11 as effectively as geraniol, but because cell type 1 does not respond at all to linalool, this odorant should be easily discriminated from geraniol by this different ratio of inputs from the two ORN types. From Anderson et al. (1995).

The mosquito maxillary palp ORNs on *A. egypti* investigated by Grant et al. (1995) were able to respond to changes, either up or down, in CO_2 concentration as low as 50 ppm, and had very steep dose-response curves in response to the even wider range of CO_2 concentrations that would occur in nature, making them very good flux detectors. However, they also could respond reliably to absolute ambient concentrations of CO_2 without adapting, and accurately reported these levels whether achieved by the rapid addition or subtraction of CO_2 (flux) or slower equilibration of background CO_2 levels. The behavioural relevance of flux responses would appear to be important for host-finding (Grant et al. 1995), but that of absolute concentration detection is less clear.

Another ORN tuned to CO_2, on the labial palps of the moth *Heliothis armigera*, also was found to exhibit both phasic and tonic firing characteristics, potentially giving it the ability to determine absolute concentrations of CO_2 as well as changes in concentration (Stange 1992). Incredibly, this ORN was sensitive to fluctuations in CO_2 levels as small as 0.14%, or 0.5 ppm. The behavioural significance of this CO_2 detection system is not clear, but the portion sensitive to fluctuations rather than absolute concentrations of CO_2 may be related to host-plant location by the adults, or perhaps to migratory behaviour related to changes in atmospheric CO_2 pressure at various altitudes (Stange 1992).

If differentially tuned ORNs are to accurately report changes in concentration as an ensemble that also maintains the accurate reporting of odour

quality, then each type must respond to changes with a similar change in rate of firing. Among other things, this means that their activity must be independent of the activities of neurons with which they are co-compartmentalised in the same sensillum. This does in fact seem to be the rule in insects, although there are a few examples of mixture interactions occurring among insect ORNs that would alter the information at the sensory neuronal level before reaching the antennal lobe (Den Otter 1977; van der Pers et al. 1980; O'Connell 1985; O'Connell et al. 1986). Studies in which mixture interactions do not occur, and activities of ORNs are not affected by those of the co-compartmentalised ORNs, include those by Boeckh and Ernst (1983) and by Akers and O'Connell (1988, 1991). Recordings of ORNs are typically if not always made at the sensillum level near the axonal spike-generating region of the cell body. It is not unequivocally known what happens to the activities of antennal neurons farther down the antennal nerve where there could potentially be interactive effects due to gap junctions or other interaxonal phenomena (Zacharuk 1980).

There are several studies that have indicated the potential for mixture interactions that could affect the accurate reporting of odorant ratios. Especially noteworthy are examples in which one neuron hyperpolarises and is inhibited when exposed to one type of odorant but is excited by a different odorant (Kaissling et al. 1989b; Hansson et al. 1990a; Todd et al. 1992). Such interactions can profoundly affect behaviour (Kramer 1992) and more such phenomena should be searched for in insects in the future.

The rarity of mixture interactions among the peripheral ORNs of insects contrasts with their prominence in the olfactory system of lobsters (Atema et al. 1989). In the American lobster, *Homerus americanus*, Atema et al. (1989) described instances in which the responses of single neurons are significantly altered by the activities of other neurons responding to other odorants blended with the original single stimulus. These interactions often reduced the action potential frequency of the neuron in question to below that which would have occurred had the other components not been added (Atema et al. 1989; McClintock and Ache 1989a; Voigt and Atema 1992; Voigt et al. 1997). Less often, the interactions in the periphery are synergistic, elevating the output over what would have occurred in response to the single odorant. Each of the primary peripheral receptor organs on the antennules of *H. americanus* houses over 140 neurons; thus, it seems that there is more potential for mixture interactions in lobster than in insect olfactory receptor organs. Insect olfactory sensilla usually house 10 or fewer neurons (Hansson et al. 1991b; Chap. 1), with the majority that have been studied housing two or three (Hansson 1995, Hildebrand and Shepherd 1997).

The sensitivity of ORNs in general has been found to vary little with environmental or physiologial changes, with the exception that the overall responsiveness of ORNs has been commonly shown to decline with age. This may be attributed to such mundane effects as desiccation and other general wear-and-tear phenomena. Two examples of increases in responsiveness should, however, be noted. Davis (1984) found that lactic acid ORNs on the antennae of female *A. aegypti* become less sensitive to lactic acid after a blood meal is taken. Conversely, following oviposition by a blood-fed female, the sensivitivy of these ORNs returns to pre-bloodmeal levels (Davis 1984). Den Otter et al. (1991) performed EAG recordings with several species of tsetse flies and showed that antennal sensitivity in both sexes of *Glossina m. morsitans* and *G. tachinoides* increased as the number of days since their last blood meal increased. Antennal sensitivities of two other *Glossina* spp. were not affected by feeding, but all four species also exhibited declines in sensitivity due to age. In both *A. aegypti* and *Glossina* spp. it is not clear the degree to which behavioural sensitivity would be increased by the increased sensitivity of ORNs, but nevertheless, clearly in some instances the overall sensitivity of olfactory pathways may be heightened by changes occurring in these primary afferents.

3.3 Co-compartmentalisation of ORNs Within the Same Sensillum

A largely overlooked aspect of insect olfaction is that insects, by possessing an exoskeleton, have the ability to compartmentalise their sensory neurons and bathe them in specialised pools of binding protein, thereby giving them the potential to possess an extra degree of olfactory resolution. Insect ORNs are housed in evaginated organs (sensilla [hairs], pegs, plates; Chap. 1) rather than in an invaginated sinus as in vertebrates. This seems to provide an extra opportunity to detect and report aspects about the fine structure of an odour plume that, in the mammalian system, would be unresoluable. In mammals, the primary ORNs are imbedded in a sheet of what appears to be contiguous, homogeneous binding proteins (Hildebrand and Shepherd 1997), designed to sample the homogenized, inhaled plume.

We propose that there are at least two advantages to co-compartmentalisation of ORNs within the same sensillum. First, it will allow for a higher fidelity of reporting of the relative ratios of odorants to which the co-compartmentalised neurons are tuned, compared with non-co-compartmentalised neurons. Second, it will allow for the highest resolution of odour strands and the highest ability to detect and report to the brain whether two or more odourants in a blend are present in the same strands or in separate, intertwined strands. This is critical for the insect's behavioural response, because the former would indicate that the odourants originated from a single point source upwind, and the latter would indicate that the odourants could only have originated from separate sources upwind.

An example of how the quality of an odour blend is better preserved by co-compartmentalisation than by the random housing of two ORN types in separate sensilla comes from the data of Akers and O'Connell (1991). They found that in the redbanded leafroller, *Argyrotaenia velutinana* (Walker), the responses of the two types of ORNs tuned to the two most critical pheromone components Z11-14:Ac and E11-14:Ac were the same whether a blend of the two components in the natural 92:8 Z:E ratio was presented, or whether the components were presented individually. The two neuronal types, one a large-spiking neuron responsive to Z11-14:Ac (the A neuron) and the other a small-spiking neuron (the B neuron) responsive to E11-14:Ac, are always paired within the same sensillum.

The data of Akers and O'Connell (1991) were originally displayed to highlight how the intensity of a single neuron's response to a single component does not vary whether the stimulation came from the component being puffed individually or as part of a blend. A rearrangement to highlight responses in individual sensilla to different blends now clearly shows the high degree of fidelity of ratio reporting from the two neurons housed within (Fig. 6). This fidelity stems from the co-compartmentalisation of the two neurons within the same sensillum. There is a high degree of variation in the absolute levels of firing rates of the A and B neurons among sensilla, which may be due to variations in the porosity of the sensillar cuticle among sensilla (O'Connell et al. 1983), or perhaps due to naturally or experimentally produced differences in the access that odorants have to the sensilla caused by the different positioning of the sensilla relative to the odourant-bearing airstream.

It can be seen that a 50:50 Z:E ratio (Fig. 6, top) generally produces a consistent 1:1 ratio of action potentials from any individual pair of A and B

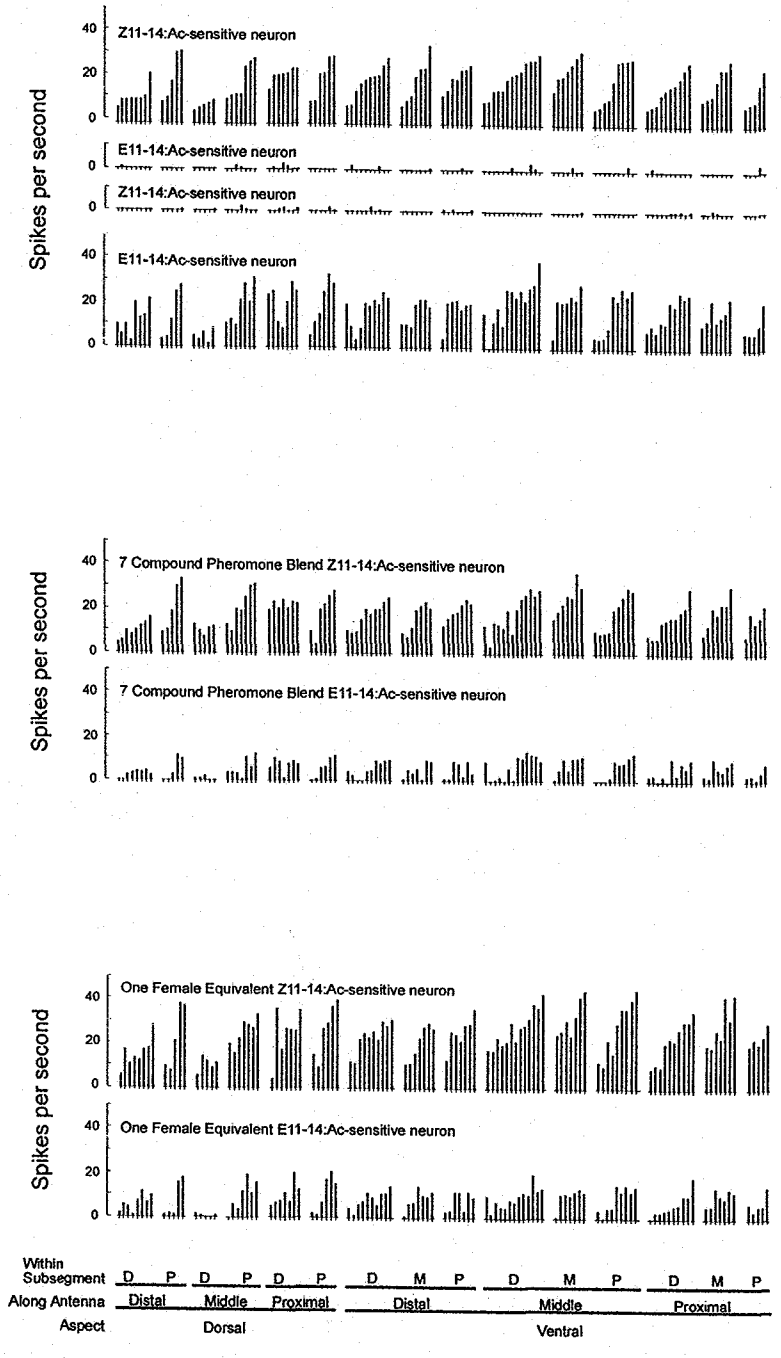


Fig. 6. Responses of A-type (large-spiking neuron) and B-type (small-spiking neuron) antennal ORNs paired within 113 sensilla trichodea on the antenna of *A. velutinana* males in response to the major and minor pheromone components in this species' pheromone, a blend of 92% Z11-14:Ac and 8% E11-14:Ac. The sensilla are arranged according to their position on the antenna, either dorsally or ventrally located, proximally or distally, and then within each subsegment according to whether they were in the proximal, middle, or distal region of the subsegment. They were then arranged within each location from the lowest to the highest firing frequencies. In the *top row*, single components were puffed over the sensilla at equal cartridge loadings. Akers and O'Connell (1988) showed that the action potential frequency of these single neurons in response to a given dosage did not vary, whether they were presented to the neurons as a blend or as single components. Therefore, the responses to 50% Z11- and 50% E11-14:Ac in the top pair is represented by an approximately 1:1 ratio of neuronal firing by the A neurons in each sensillum tuned to Z11-14:Ac and the B neurons in the same sensillum tuned to E11-14:Ac, regardless of whether the neurons in an individual sensillum were more, or less, sensitive to the stimulus. The ratios of firing in response to the natural 92:8 blend of Z11- and E11-14:Ac, either presented as a synthetic mixture (*middle*) or gland extract (*bottom*) were now consistently at approximately a 3:1 ratio, regardless of the neurons' absolute sensitivity (firing frequency) in response to these components. From Akers and O'Connell (1991)

neurons within a sensillum, regardless of whether their absolute levels of firing are high or low within that sensillum. In contrast, a puff from cartridges emitting the natural 92:8 Z:E ratio generally produces a consistently different ratio – a 3:1 ratio of firing from the A and B neurons, respectively, in any given sensillum (Fig. 6, bottom), regardless of the pair's absolute sensitivities (firing rates) to these components. The high fidelity of this 3:1 firing ratio in response to the 92:8 Z:E blend is maintained within a sensillum even when the five other components are added to the blend; the gland extract with all seven compounds present results in the same within-sensillum firing ratio (Fig. 6, middle) as when the synthetic two-component blend is presented (Fig. 6, bottom).

The pattern seems to be that for those species and situations where blend ratio discrimination for a particular semiochemical blend is very high and is critical for optimal behaviour, the ORNs tuned to these compounds are found to be co-compartmentalised rather than individually housed in separate sensilla. Such neuronal pairings are found in both strains of *Ostrinia nubilalis* (Hübner) (Hansson et al. 1987; Roelofs et al. 1987; Cossé et al. 1995), in *A. velutinana* (O'Connell 1975; Akers and O'Connell 1988, 1991), in *G. molesta* (Baker et al. 1988), and in species of the genus *Yponomeuta* for which the ratio of Z11- to E11-14:Ac is critical (van der Pers and den Otter 1978; van der Pers 1982; van der Pers and Löfstedt 1986; Löfstedt et al. 1990). In the above instances, often only a 1–3% change in blend ratio is needed to cause a significant change in behaviour.

Conversely, when blend ratios are not known to be as critical for optimal behavioural response, ORNs are more often not paired within the same sensilla. Examples include *A. segetum* (Löfstedt et al. 1985, 1986; Hansson et al. 1990b), *Trichoplusia ni* (Hübner) (O'Connell et al. 1983, Grant and O'Connell 1986), *Pseudoplusia includens* (Walker) (Grant et al. 1988), *H. zea* (Almaas et al. 1991, Cossé et al. 1998), *Heliothis assulta* (Berg and Mustaparta 1995), and *H. virescens* (Berg et al. 1995, Hansson et al. 1995). At least 10–100% changes in ratios must usually occur before a significant change in the behavioural responses of these insects occurs.

The optimisation of blend-quality discrimination is only one advantage of co-compartmentalisation of ORNs. There has been emerging evidence that insects can discriminate between two overlapping odour plumes due to the incomplete mixing of their intertwined filaments (Witzgall and Priesner 1991, Liu and Haynes 1992). Wind-tunnel bioassays using experimentally generated, pulsed plumes have demonstrated that two odorants are not as effective when they are presented in staggered fashion (in every other pulse) compared with when they are coincident in every pulse. This is true whether the odorants are a blend of two pheromone components (Vickers and Baker 1992) or a two-component pheromone blend to which a behavioural antagonist is added (Fadamiro and Baker 1997, Vickers and Baker 1997).

The most recent results provide even more startling information about the degree of fine-grained resolution abilities of the moths. *H. zea* males can distinguish completely coincident strands of pheromone and antagonist from those whose strands are separated by only 1 mm (Baker et al. 1998). Baker et al. (1998) pointed out that co-compartmentalisation of the neurons that are differentially tuned to these odorants (Cossé et al. 1998) should optimise both the spatial and the temporal resolution ability of the animal.

For male insects, the ability to discriminate their conspecific pheromone strands from the strands of a second, non-conspecific female whose plume overlaps the conspecific female's, could have real fitness advantages in allowing males to not be erroneously deterred by the presence of antagonistic compounds in a plume and to successfully locate and mate with their own females. The key would be whether the antagonist strands are not completely coincident with the pheromone strands, which could only arise from there being two different sources, one being the conspecific female. If all of the strands were completely coincident, the strands can only have originated from one source. For three different moth species, two point-source plumes – one an antagonist and the other a pheromone blend – placed only centimetres apart laterally or longitudinally along the windline, have been found to be sufficiently separated that male moths can still locate the pheromone source (Miller and Roelofs 1977; Witzgall and Priesner 1991; Liu and Haynes 1992, 1994). In two of these species, *T. ni*, and the gypsy moth (*Lymantria dispar*), in which sensory neuronal recordings also have been performed, the neurons tuned to the major pheromone component and to the antagonist are co-compartmentalised in the same sensilla (O'Connell et al. 1983; Hansen 1984; Grant and O'Connell 1986; Todd et al. 1992).

The gypsy moth and the nun moth (*Lymantria monacha*), share the (+) enantiomer of disparlure as their key pheromone component, with gypsy moth attraction being antagonised by the presence of the (–) enantiomer (Miller and Roelofs 1977). Sensilla on *L. dispar* antennae contain a large-spiking neuron sensitive to (+) disparlure and a small-spiking neuron tuned to (–) disparlure (Hansen 1984). Gypsy moth males can locate sources of (+) disparlure in an overlapping plume of (–) disparlure, even separated laterally by only 10 cm, but not as readily when it is co-emitted from the same source as (+) disparlure (Miller and Roelofs 1977). The (–) enantiomer has no discernable antagonistic or agonistic effect on the nun moth, and there is only one large-spiking neuron in the sensilla of *L. monacha* males responding to both the (+) and the (–) disparlure. Co-comparmentalisation of an antagonist-responding neuron with that of a pheromone component also occurs in, among other species, *A. segetum* (van der Pers and Löfstedt 1986), *O. nubilalis* (Hansson et al. 1987, Roelofs et al. 1987, Cossé et al. 1995), *H. assulta* (Berg and Mustaparta 1995), and *Y. rorellus* (Löfstedt et al. 1990).

For mere fine-grained blend-ratio quality resolution, it should not be necessary for the information present uniquely in each pair of co-compartmentalised neurons to be retained and assessed by the CNS. All that would be needed is for them to covary due to their being immersed in the same receptor environment. The average of these covarying neurons from the whole antenna should still give as accurate a rendering of the blend ratio as when individual ratios are preserved. However, discriminating between the difference of the arrival of one versus two odour strands in either time or space would seem to require that the local stimulus situation at each sensillum be processed on-site by the neurons within the sensillum before being sent to the CNS. A second possible mechanism would be to send the information from each pair of co-compartmentalised neurons all the way to the antennal lobe without processing, and then have the CNS integrate each pair of arriving inputs separately but simultaneously. Either way, the information from each antennal site (hair) should then be compared with others, for temporal or spatial differences. As yet, there is little evidence that the AL preserves topographic information from the antenna with the high degree of site specificity needed to explain this level of spatial or temporal resolution (Christensen et al. 1995b, Hildebrand and Shepherd 1997). There has, however, been an indication that there may be much more on-site mixture processing involving hyperpolarisation and inhibition within olfactory sensilla in insects than has been realised that could provide the potential for stimulus suppression that would account for this high degree of olfactory resolution.

Co-compartmentalisation perhaps provides an extra challenge for the biochemical machinery in the receptor–perireceptor environment, in that two different kinds of molecules, often with different functional groups or double bond positions, must be ferried across the sensillar lymph to their respective membrane-bound receptor sites. Is this done by a common binding protein or two different ones? The behavioural end result could be dramatically different for the two perireceptor–receptor systems within the hair, and interesting questions such as whether the odourants themselves are receptor ligands or the binding-protein-odourant complexes are receptor ligands (Chap. 2) might be ideally experimentally addressed using co-compartmentalised agonist- and antagonist-tuned neurons.

Very fine-grained resolution of odour strands should not be expected in all, or even most, insects, but it appears to be a capability emerging as being important to blend-quality resolution. There may be no need for such fine-grained resolution in mammalian systems because any fine-scale structure present in the odour plume may be lost upon inspiration. However, the issue of odour-plume structure resolution should be more closely examined in mammals because there may be more fine-grained information than we think that is preserved during sniffing.

3.4 Adaptation and Disadaptation

Insect olfactory neurons display significant differences in the duration of their responses to odorants (Dethier 1972; Kaissling 1974, 1987; Almaas et al. 1991; Berg et al. 1995). They typically exhibit a phasic-tonic temporal profile of response, with the first portion of action potential output being a sharp burst that is shorter than, and does not correlate with, the longer-lasting stimulus (Kaissling 1974, 1987). The remainder of the response is a tonic, residual low level of above-background firing that may not mirror the stimulus cessation, especially at the highest dosages (Kaissling 1974, Berg et al. 1995). The lack of correspondence with stimulus pulse presentation of both the phasic and tonic portions of the ORN response have been shown not to be artifacts resulting from the techniques used to deliver the odorants. There are examples of both highly phasic and highly tonic neurons in the same species responding to the same odorants. For example, Berg et al. (1995) found that the ORNs on male *H. virescens* antennae that were sensitive to (*Z*)-9-tetradecenal (Z9-14:Ald) responded in a more phasic fashion that those sensitive to (*Z*)-11-tetradecenal (Z11-14:Ald). Rumbo (1983) and Rumbo and Kaissling (1989) found differences in the durations of response of antennal neurons tuned to different components of the sex pheromones of the light brown apple moth, *Epiphyas postvittana* (Walker) and *A. polyphemus*, respectively. For both moths, differences were related to the type of neuron, with the neuron tuned to the major component being the more phasic, and the neuron tuned to the minor component firing more tonically.

The propensity of insect ORNs to fire in a phasic versus a tonic pattern is related to their relative rates of adaptation and disadaptation (Kaissling 1987). In general, phasic neurons are fast to disadapt and are able to follow (respond to) the presence of pheromone that is repeatedly and rapidly pulsed at up to 10 Hz (Almaas et al. 1991). More tonically firing neurons are slower to disadapt, sometimes taking minutes or tens of minutes to do so, and cannot respond to a new pulse of pheromone during this time (Rumbo 1983, Rumbo and Kaissling 1989). Even for fast adapting–disadapting ORNs, disadaptation is not necessarily absolute, and the tracking response to rapidly repeated stimuli does not include accurate information about the concentration. The number of action potentials in response to each pulse, reflecting the concentration of odorant in each pulse, is significantly reduced under rapid stimulation, but the fidelity of response with regard to the temporal presence or absence of the pheromone is accurate. Thus, the sensitivity of a receptor unit in response to an odorant is not absolute and is subject to adaptation–disadaptation mechanics (Kaissling 1986, 1987, 1990).

Contact with the strands of odorant within a natural plume will be on a highly irregular basis in time, as shown both from the work of Murlis (1986)

by using an idealised receptor (ion detector) and odour mimic (ionised air) as well as from the recordings of real odour plumes in the field and laboratory by using insect antennae as detectors (Baker and Haynes 1989). The adaptation–disadaption characteristics of ORNs are concentration and time dependent (Kaissling 1986, 1987; Almaas et al. 1991; Berg et al. 1995), and these two aspects will affect the ORNs' abilities to report to the CNS the presence of all of the strands of odourant that are contacted in a natural plume. A form of temporal averaging (Murlis 1986, 1997) of the fine structure of an odour plume would thus occur when, for instance, two equally concentrated strands contact the ORN too rapidly relative to the disadaption rate, resulting in the second strand evoking no action potentials. Alternately, contact with a low-concentration strand could follow a high-concentration strand after an appropriately long time interval, but due to adaptation from the highly concentrated first strand, again evoke no action potentials. The adaptation-disadaption-related propensity of the ORN to adjust to both concentration and frequency will thus ensure that the CNS will receive relatively unvarying inputs over a wide dynamic range of both concentrations and turbulence-induced plume structures. The integrative mechanisms in the CNS (e.g., for odor-quality discrimination and enhancement of phasic inputs (Christensen and Hildebrand 1988, Christensen 1989) will thus be buffered from outside variance in the plume, and behavioural response will likewise attain a higher fidelity over such differences in conditions.

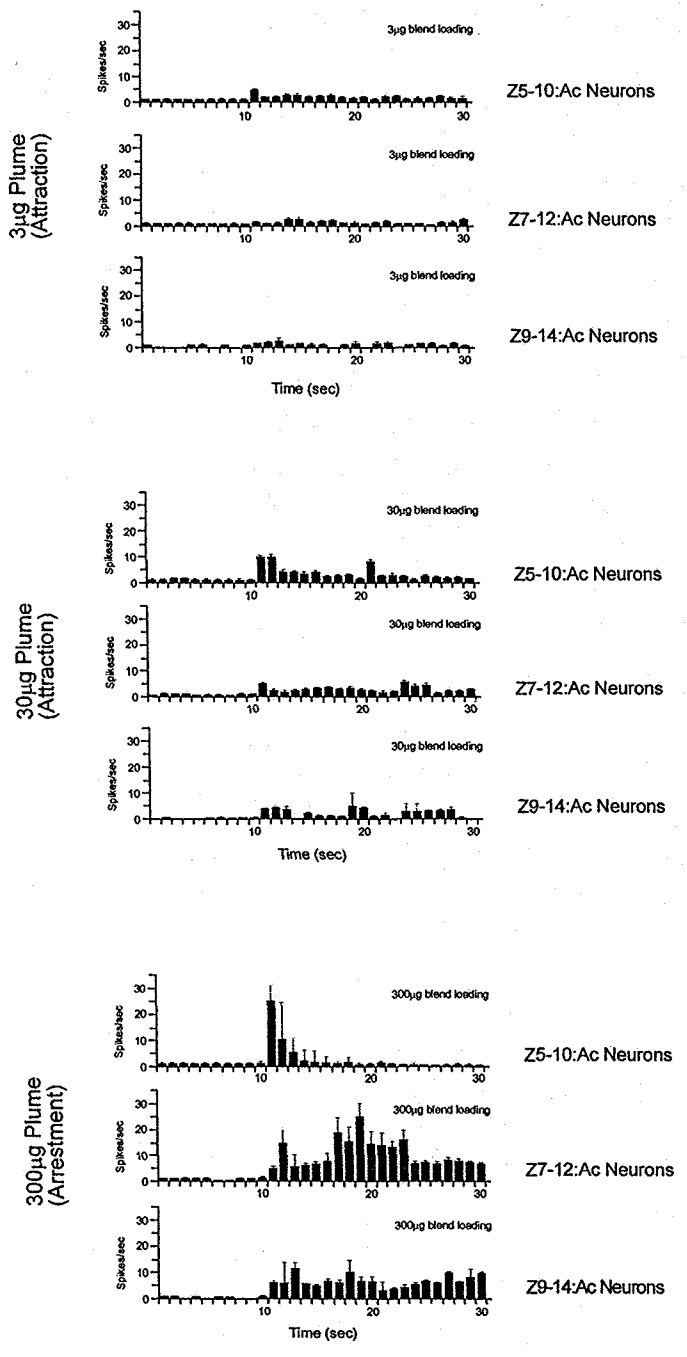
When changes in either the concentration or in the adaptation–disadaptation rates are experimentally made, such that the ability of only one type of the many ORNs to respond is differentially affected, then the discrimination of odour quality and tempo of encounters will be impaired. Exceeding the dynamic range differentially of only one type of ORN this way has been shown to be important in natural pheromone plumes and has been correlated with changes in behaviour (Baker et al. 1988). A lowering of the ambient temperature impaired the ability of antennal neurons to fire reliably in response to rapid, repeated puffs of pheromone in the oriental fruit moth. These sensory neuronal changes were correlated with the observed increase in arrestment of upwind flight under cooler compared with warmer temperatures at a given pheromone concentration and ratio (Linn et al. 1986, Linn and Roelofs 1989). Similarly, excessive concentrations of the three-component pheromone blend of *A. segetum* were shown to differentially impair the ability of only the (Z)-5-decenyl acetate (Z5-10:Ac) ORNs to disadapt and fire in response to repeated contact with filaments in a natural plume (Hansson and Baker 1991). The competency of neurons tuned to both Z7-12:Ac and Z9-14:Ac to fire in this very same plume was completely unaffected (Fig. 7). Thus, the excessive plume concentration caused a distorted ratio of firing from the three ORN types, with now only two types firing when there should be three. The

responses of all three neuronal types to either 10- or 100-fold lower pheromone source loadings were correlated with high levels of completed upwind flights to the source. Both Z7-12:Ac and Z9-14:Ac would be emitted at lower concentrations than Z5-10:Ac due to their lower volatility, and this would explain in part the ability of the ORNs tuned to these two components to avoid becoming adapted to the very same frequency of filament contacts that produced adaptation of the Z5-10:Ac-tuned neuron (Fig. 7; Hansson and Baker 1991).

Intermittency in the odour stimulus has been shown to be extremely important for evoking certain behaviours such as sustained upwind flight or walking to an attractant source (Kennedy et al. 1980, 1981; Baker and Kuenen 1982; Kuenen and Baker 1983; Baker 1985; Kramer 1986, 1992). The importance of a phasic response of peripheral neurons to sustained upwind movement comes from direct experimental manipulation of their outputs (Kaissling et al. 1989b; Kramer 1992). In a clever series of experiments, Kaissling et al. (1989b) found that an analogue of bombykol, the pheromone of the silkworm, *Bombyx mori* (L.), caused highly tonic firing of the antennal ORNs tuned to bombykol, lasting several minutes, and that this tonic response was correlated with no upwind walking toward the source. Kaissling et al. (1989b) also found that the plant-related compound linalool effectively hyperpolarised this neuron and prevented action potentials from occurring whenever it was puffed over the sensilla. When they first stimulated the ORNs with the pheromone analogue to induce high levels of tonic firing, and then interrupted the firing with intermittent puffs of linalool, they could create repeated, regular bursts of firing from the bombykol neurons that was indistinguishable from repeated puffs of bombykol itself. They reasoned that this highly artificial stimulus that does not contain the pheromone but mimics a reiterative pattern of phasic output from the pheromone-sensitive neuron, should be a behaviourally effective stimulus that should result in upwind walking by intact male moths, and Kramer (1992) found that this was indeed the case. High levels of upwind walking to the source occurred in response to this unnatural stimulus.

4 Evolution of Peripheral Olfactory Organs

Mutations in insect olfactory communication systems have rarely been discovered or studied. John Carlson and colleagues (Carlson 1996, deBryune et al. 1997) found that mutations in ORNs of *Drosophila* are correlated with changes in olfactory-mediated behaviour. These results and others in the area of the neurogenetics of olfaction are discussed thoroughly in Chap. 10. The *Drosophila* findings show that neuronal mutations can involve the silencing



of a neuron, in addition to the altering of the response spectra of other neurons (deBryune et al. 1997). These mutations were discovered after behavioural evidence pointed the way to examining olfactory pathways for possible changes.

In *T. ni*, a mutation in the pheromone emission system was discovered in a laboratory colony that included a 20-fold over-emission of the minor component Z9-14:Ac compared with wild-type females (Haynes and Hunt 1990), but no significant corresponding behavioural changes (Haynes and Hunt 1990) or alterations in the antennal ORN population (Todd et al. 1992) were found in the initial populations of mutant males. Pure mutant-type males continued to discriminate for the wild-type blend over the mutant blend, just as wild-type males, and responded very poorly to the mutant blend (Haynes 1997). However, after 31 generations of rearing in a pure mutant colony, pure mutant-type males finally evolved to respond as well to the mutant blend as they did to the wild-type blend (Liu and Haynes 1994; Haynes 1997). After this evolution in behaviour to accept a wider range of proportions of Z9-14:Ac and Z7-12:Ac, the ORNs tuned to Z9-14:Ac were reexamined, and they appeared now to be reduced in number and in sensitivity to Z9-14:Ac, which could conceivably diminish the behavioural sensitivity of the moths to this overabundant component (JL Todd, KF Haynes, AA Cossé, and TC Baker, unpubl. data). The mutant blend might now produce a pattern of inputs from the ORNs that is more like the pattern produced by the normal blend and could account for the behavioural acceptance of the mutant blend by mutant males. There also may be CNS changes that would account for the greater tolerance for a wider set of blend ratios exhibited by these males, and this mutant *T. ni* system certainly deserves further examination.

Fig. 7. Responses of three types of differently tuned ORNs on the antennae of *A. segetum* in response to natural point-source plumes of the three-component sex pheromone blend of this species at three different concentrations. The three types are those tuned to Z5-10:Ac (1-s average responses from 33 neurons, *top* in each trace), Z7-12:Ac (1-s averages from seven neurons, *middle*), and Z9-14:Ac (1-s averages from two neurons, *bottom*). None of the neurons respond to other than the component they are tuned to, at any dose. The tracings show the responses during a 10-s prestimulus period, and the firing rates increase beginning at 10 s into each tracing when the plume was introduced into the wind tunnel and allowed to flow over the single-cell preparation. At the low and mid-range source dosages (3 µg and 30 µg), all three types of neurons respond to the plume for the entire 20 s of exposure to the plume, and thus would report the presence of the complete, 3-component sex pheromone blend to the antennal lobe of the brain. In behavioural experiments these two point source dosages promoted complete upwind flight to the source by males. The highest dosage of the 3-component blend (300 µg), which caused arrestment of upwind flight in behavioural experiments, caused the Z5-10:Ac-tuned ORNs on the antennae to adapt after only ca. 5 s of exposure to the plume, and to cease firing. The ORNs tuned to Z7-12:Ac and Z9-14:Ac continued to accurately report the presence of these two components in the plume for the entire 20-s period. From Hansson and Baker (1991)

That the ORN systems of insects can change over time is graphically illustrated by the findings of George and Nagy (1984), who showed that after 20 years of rearing the same culture of oriental fruit moth, the number of sensilla trichodea housing pheromone RNs on male antennae was reduced to one-half of the original, wild-type number. There is evidence for at least one noctuid moth species, *A. segetum*, that when there is variation in the abundance of pheromone components across a geographical range, the ORN system mirrors this variation (Löfstedt et al. 1986, Hansson et al. 1990b). In *A. segetum*, the proportion of ORNs tuned to Z5-10:Ac, Z7-12:Ac, and Z9-14:Ac is at least coarsely reflected in the proportions of these compounds found in gland extracts of females (Löfstedt et al. 1986; Hansson et al. 1990b; Löfstedt 1990). If the vapor pressure of the components were to be considered, however, the degree of correspondence between *emitted* proportions of components and proportion of ORNs tuned to these components would certainly be more highly correlated. For noctuid moths in general, the ORNs that are tuned to the less abundant components in the emitted blends are themselves less abundant (Almaas et al. 1991; Todd et al. 1992; Berg et al. 1995; Berg and Mustaparta 1995; Hansson 1995; Hansson et al. 1986; Cossé et al. 1998). This is the opposite of what is to be expected if sensitivity optimisation is the reason for the relative abundance of ORN types.

For crambid, tortricid, and yponomeutid moth species for which blend ratios are critical, the ratio of ORNs tuned to the critical components does not vary with species or races. Rather, they remain at a 1 : 1 ratio to each other and are always paired with each other in the same sensilla. Co-compartmentalisation could help improve discrimination of blend ratio. One relationship that does seem to occur predictably among races or species is that the sizes of the action potentials of the neurons correspond to whether they are tuned to the more abundant (major) or the rarer (minor) component. In the E race of the European corn borer, the large-spiking neuron is tuned to the major component E11-14:Ac and the small-spiking neuron is tuned to the minor component Z11-14:Ac. In the Z race it is just the opposite, with the large-spiking neuron tuned to Z11-14:Ac, the major component in the pheromone blend of this race (Hansson et al. 1987, Roelofs et al. 1987). Why this should be so is again unknown, but because the large-spiking neuron is slightly more sensitive to its component than the small-spiking neuron (Cossé et al. 1995), and dendritic size seems to be related to spike size (Hansson et al. 1994b), there may be a larger number of receptor sites available to detect the major pheromone component, thereby increasing neuronal sensitivity. Again, it would seem as though the sensitivity to the minor component would need to be amplified rather than vice versa, so some other relationship may be occurring relating to the genetics governing ORN architecture.

In yponomeutid moth species, there is also a correspondence between large- and small-spiking neurons and whether the component to which they

are tuned, Z11-14:Ac or E11-14:Ac, is the major or the minor component in the blend (van der Pers and den Otter 1978; van der Pers 1982; van der Pers and Löfstedt 1986). However, in addition, blend discrimination in a third olfactory dimension is created by the existence of neurons sensitive to behaviourally antagonistic components emitted by other sympatric yponomeutid species (Löfstedt and Van der Pers 1985, Löfstedt et al. 1991), as well as by the agonistic effect of these compounds on the species emitting them. The presence of neurons tuned to antagonistic compounds is evidence for an adaptive response to the blends of non-conspecifics during evolutionary shifts in blend ratio (Löfstedt 1990, 1993; Phelan 1997), rather than an incidental shift occurring as a result of a specific mate-recognition system (Paterson 1985, 1993). The adaptive shift in pheromone blends would not need to be a reinforcement event occurring only during speciation. Rather, it could occur during reproductive character displacement when two species with similar blends find themselves in sympatry (Löfstedt 1990, 1993; Butlin 1987). Such neurons can be found in many species of moths, and they are nearly always paired with neurons sensitive to pheromone components within the same sensilla.

Many neurons tuned to antagonists now appear to be tuned equally to several such compounds emitted by other species. That there should be such broad tuning is interesting and could indicate that less specifically tuned receptors allow for important shifts in behaviour, and in olfactory discrimination over evolutionary time. Neurons also exist that are broadly tuned for agonistic compounds as well, and again these types of neurons could facilitate shifts in blend emissions by females due to reproductive character displacement. In *H. zea*, the neuron tuned to the minor pheromone component Z9-16:Ald is equally sensitive to Z9-14:Ald (Cossé et al. 1998) and appears to allow for a positive behavioural response to either, when they comprise the second component in a two-component blend (Christensen et al. 1991, Vickers et al. 1991).

In *Y. rorellus*, the neuron tuned to the major pheromone component 14:Ac is also equally responsive to several other compounds, such as (*E*)-6-tetradecenyl acetate (E6-14:Ac) and (*E*)- and (*Z*)-12-tetradecenyl acetate (E12- and Z12-14:Ac), none of which is emitted by any known yponomeutid species, in addition to being nearly equally responsive to the major components (E11- and Z11-14:Ac) of several other sympatric yponomeutid species (Löfstedt et al. 1990). Were it not for the fact that there is a neuron tuned to known antagonistic compounds that responds with high sensitivity to these major yponomeutid components as well, *Y. rorellus* males would fly upwind in response to females of other species. They are highly attracted to E6-14:Ac, and E12- and Z12-14:Ac, none of which are pheromone components but all of which stimulate only the 14:Ac-sensitive neuron and not neurons sensitive to antagonists. Thus, male *Y. rorellus* only fly upwind and locate their own females that emit the single component 14:Ac, and the unusual, broadly tuned

ORN may have allowed for this unusual pheromone component (14:Ac) to arise and be favoured in the context of reproductive character displacement that perhaps has occurred in this group of sympatric yponomeutid species (Löfstedt et al. 1990).

5 Concluding Remarks

Peripheral olfactory receptor organs in insects provide an interesting glimpse into the selection pressures that have moulded the behavioural responsiveness of insects over evolutionary time. As highly accessible, evaginated hairs or as knobs or plates on the evaginated antennae, they protrude into the environment to sample odours in their natural state – in plumes in moving air. They are capable of providing the brain with a rendering of the composition of single odour filaments, and of the fine-grained structure of overlapping plumes that have originated from two different sources. Peripheral ORNs of insects can provide odour-quality information reliably over a large range of concentrations of a large array of odour blends with high sensitivity. The rendering of all of these odour plume characteristics is accomplished by means of a relatively simple set of neuronal response characteristics of excitation and inhibition by differentially tuned units, some of which apparently report to networks in the antennal lobe that are associated with agonistic behavioural outcomes, and others that are associated with antagonistic outcomes. The tension between agonistic and antagonistic systems should result in the very fine blend discrimination that insects exhibit for the many types of odorants that are important in their lives.

Future work should include more investigations of the possible effects of blend interactions on the activities of ORNs. Advances in understanding the limits of olfactory resolution, both spatial and temporal, may be made in the course of such investigations. Other advances can be made by more carefully quantifying the stimuli presented to ORNs. A starting point for improving this aspect of studying insect olfaction is to find easier, more sensitive ways to quantify the amounts emitted from odour cartridges. Finally, studying mutant and hybrid individuals' ORNs will provide insights into how changes in the response spectra of ORNs can impact behaviour and contribute to the evolution of new kairomone, pheromone, or allomone systems.

CHAPTER 4

Antennal Lobe Structure

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Contents

1 Introduction

Primary olfactory brain centres are unique in anatomical organisation throughout the animal kingdom and can be easily recognised by their spheroidal neuropilar subcompartments termed olfactory glomeruli. This chapter reviews the anatomical organisation of the first-order olfactory brain areas in insects, the antennal lobes (ALs). The ALs are part of the deutocerebrum of