THE CUTTING EDGE OF

Insect Olfaction

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Odor discrimination by insects can be exploited by entomologists to make species-specific pheromone traps and mating disruptants or to manipulate insects via host odors. Recent neuroanatomical studies have revealed how odor blends are represented by antennal neurons and their associated target glomeruli in the first layers of the brain as the relative abundance of each individual component in the blend. Further processing by neurons deeper in the brain decodes these lines of component-specific information and integrates them to produce enhanced signals that represent the blend quality. This article describes techniques that have aided in tracing and mapping olfactory pathways, thereby improving our ability to design new strategies for short-circuiting odor-mediated insect behavior.

THE PAST SEVERAL YEARS HAVE SEEN RAPID and exciting progress in understanding animal olfaction in both vertebrates (humans and rats) and invertebrates (insects and lobsters). However, it has been entomologists working on sex pheromone systems who have led the way toward a new level of understanding of how olfactory information is processed, and these basic principles now appear to be shared by both vertebrates and invertebrates. Both kinds of animals decode the information carried in an odor blend by breaking it down into its component parts within receptor organs and reassembling it in higher brain centers. In vertebrates, odor molecules are intercepted by cilia extending into the mucous membrane of tissues located in the nose; in invertebrates, they are intercepted by cuticular "hairs" on the antennae that contain a similar mucuslike gel that bathes the dendrites of sensory neurons. Each component of an odor blend may be recognized by a specific type of sensory neuron in the nose or antennae. The axons (the part of a neuron that extends into the brain) from odorant-specific sensory neurons seem to converge in localized regions of the brain (olfactory bulb in vertebrates and antennal lobes in invertebrates) called glomeruli. The pattern of activity in the various glomeruli defines the odor. Here we present a story of how structure and function are beautifully wedded in the architecture of the antennal lobes of insects, a relationship that plays a major role in odor-mediated behaviors, with an amazingly similar sequel also having been penned by researchers on vertebrate olfactory systems.
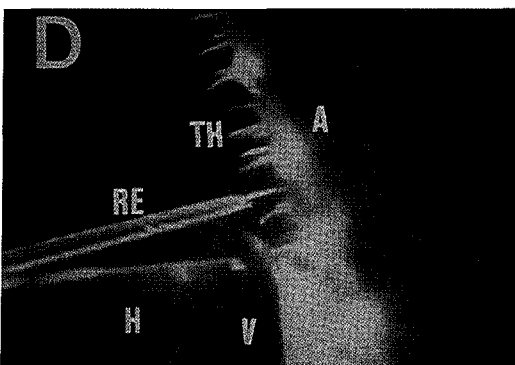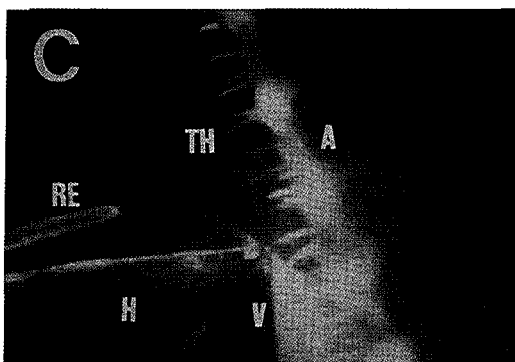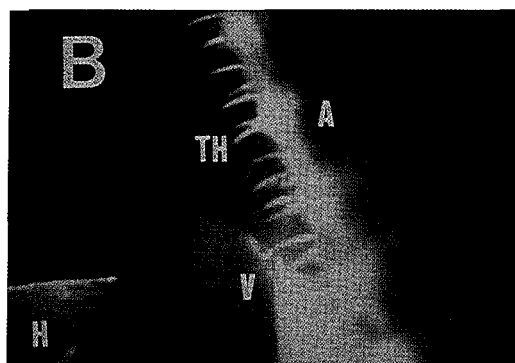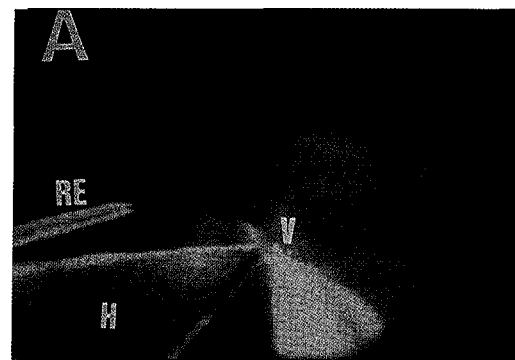
It is the thousands of extra-long receptor hairs on the antennae of male moths that first receive the information carried in the tendrils of an airborne plume of odor molecules released from the female moth that may be calling (releasing pheromone) many tens or hundreds of meters away. On and within these hairs, initial olfactory contact between male and female thus occurs, and the male becomes behaviorally active and responds by flying upwind to the female, reducing the distance between them. Evolutionarily, it is where the rubber meets the road in terms of mating success, and the molecules sticking to these hairs turn on two programs of anemotaxis (steering with respect to the wind by using the motion pattern of cues from the visual field) and counterturning (zigzagging flight) that together surge the male forward and bridge the gaps between the wisps of airborne odor in the shifting wind, driving the male's genes onward toward the female.

The knowledge gained from neurophysiological studies of moth olfaction that we outline here is particularly exciting because it meshes so nicely with our understanding of upwind flight behavior in moths. Pheromone
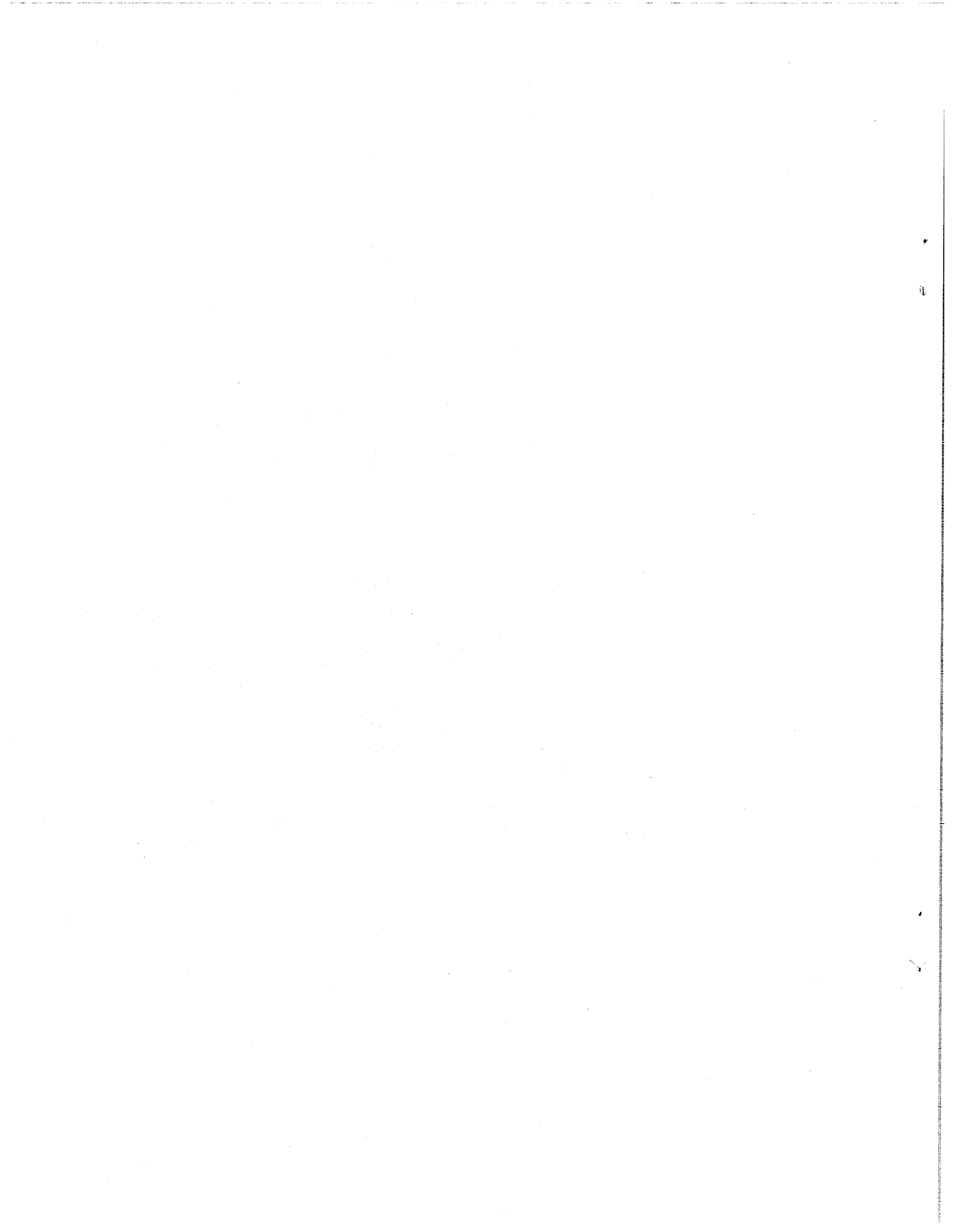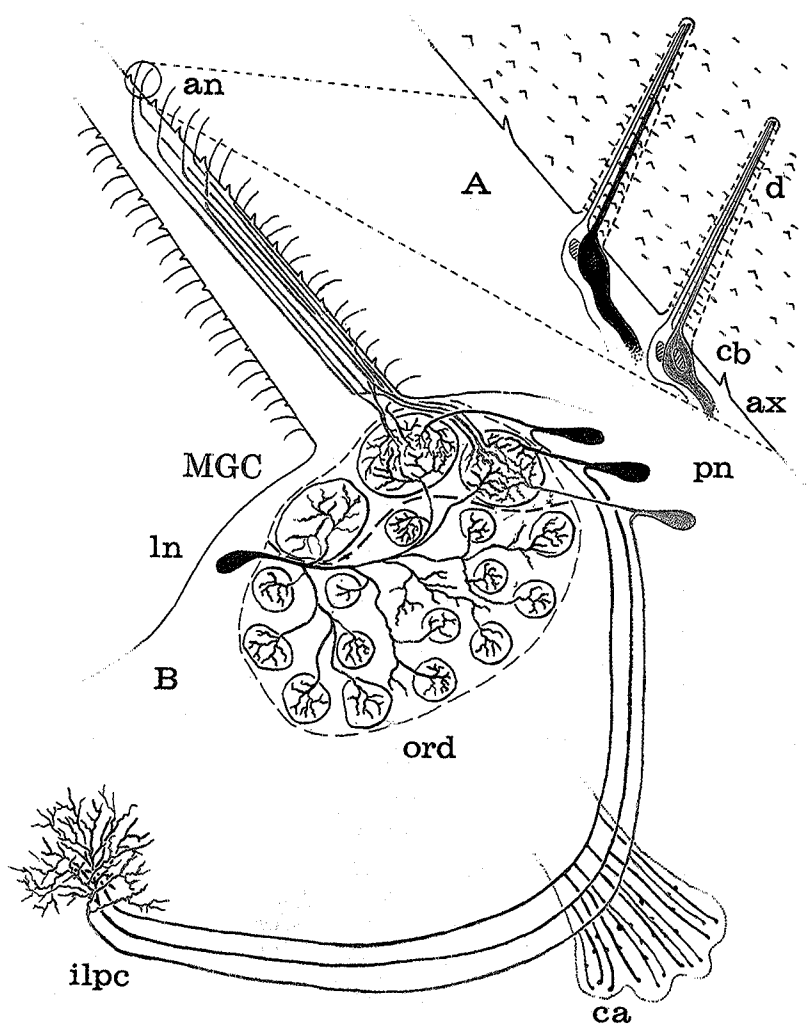
travels downwind in plumes that have fine-scale structure consisting of concentrated strands of pheromone and pockets of clean air. It is just as behaviorally important for a male moth to respond to the pockets of clean air between pheromone filaments as it is to respond to the pheromone filaments themselves because clean wind no longer points toward the source, and a male continuing upwind for too long upon flying into clean wind can find itself flying off-line from the source. Therefore, getting the excitation (nerve impulses) caused by a pheromone filament to turn off quickly so that the male can change its behavior at the first hint of clean air is very important. Neuronal inhibition that accentuates the first loss of pheromone (i.e., the first onset of clean air) thus functions to sharpen olfactory acuity, both with regard to blend quality and behavioral response time (Baker 1990). Turning off the nerve impulses quickly also is important, even if the pocket of clean air is only a small one comprising the fine-scale structure of the plume. The pheromone-blend olfactory pathways need to be made ready to detect the arrival of the next filament because each filament plus wind serves as an indicator of source direction. An upwind flight surge in response to each filament will, therefore, advance a male directly toward the source, but if its olfactory pathways have not recovered sufficiently after a filament contact, and it is unable to detect the next filament in line, it will miss important information that could have helped it advance faster (Vickers and Baker 1995).

The coupling of the nervous system to behavior begins when pheromone molecules released from a female moth land on the surface of a conspecific male's antennal hairs. The pheromone molecules enter the interior of these hairs through pores and associated tubules and traverse the gel comprised mainly of pheromone-binding protein until they arrive at the proteinaceous receptor sites that are bound to the dendritic membrane of the pheromone receptor neuron. The dendritic portion of the neuron is located within the cuticular shaft of the hair, and the cell body is located at its base. The axonal portion of the neuron extends from the base of the cell body and projects into the brain. A depolarization of the dendritic membrane results if the pheromone component molecule has the correct geometry and electron distribution due to various double bonds and functional groups (e.g., acetate, alcohol, aldehyde, or ketone) to allow it to fit optimally into the protein pocket that is the receptor. If the depolarization is strong enough, action potentials (nerve impulses) are generated at the base of the cell body and travel down the axon toward the brain. It is at this peripheral, antennal level that the earliest advances were made by neurophysiologists studying the responses of such neurons to identified pheromone components. Of course, it was the identification of the first pheromone components and their unparalleled specificity and reliability of behavioral

(A) Two glass knives are needed to cut a sensory hair in the cut-sensillum technique, both of which are viewed under a stereo microscope at 320× magnification. One knife (V) is stationary and oriented vertically in some type of sturdy holder, such as a vise, and the other (H) is mounted horizontally into a micromanipulator that will allow it to be moved in three dimensions. (B) The antenna is maneuvered until a single trichoid hair (TH) lies across the vertical knife blade, which acts as a chopping block upon which the hair is to be cut. (C) The horizontal knife is used to cut off the tip of the hair by using a series of motions facilitated by a joy-stick; note the portion of the hair on the undersurface of the horizontal knife blade. (D) A recording electrode (RE) that contains saline or a dye is placed parallel to the horizontal knife so that it can be moved quickly into contact with the hair tip after the cut has been made. By puffing odor-bearing air over the antenna, it then is possible to determine the specificities of individual neurons that are located within the cut hair. Neurons often can be distinguished visually by spike amplitude alone, and spike analysis programs are available for analyzing the spike waveforms and providing further information about the number of neurons within the sensillum.

(A) Depiction of two of the thousands of sensilla trichodea on a male moth antenna that contain neurons tuned to each of two components of the sex pheromone emitted by conspecific females. In this hypothetical two-component 50:50 blend, the molecules from one component (for instance, blue) are able to adsorb to both hairs but can only be transported through the sensillar lymph via binding proteins in one of the hairs to arrive at and activate receptor sites specific for their particular molecule. The molecules from the other component (red) likewise adsorb to both sensilla and enter their pore tubules but only make it to receptor sites in the second sensillum due to the same binding protein transport and the specificity of the receptor sites to that component in that sensillum. (B) Integration of component-specific information coming from the antenna in the hypothetical two-component pheromone system depicted in (A). The axons from the antennal neurons tuned to component 1 (blue) arrive in their particular glomerular subcompartment in the macroglomerular complex (MGC) (represented in this figure as three large ovals at the base of the antenna) as do the axons from neurons specific for component 2 (red). The antennal neurons synapse with local interneurons (only one is depicted here, in purple) in the MGC, which can either integrate the component-specific information into a blend response, depicted here in purple, or else transmit component-specific responses (not depicted). Projection interneurons synapsing with the local interneurons only in subcompartment A or B, and perhaps directly with antennal neurons, also are depicted here as being either of a blend-integrating type (purple) or as component-specific types (red or blue, respectively). These interneurons are shown projecting to the back of the brain into the calyx of the mushroom body (ca) and then continuing around to the inferior lateral protocerebrum (ilpc). Several other types of projection interneurons are not depicted here, for example those that synapse with interneurons in the lateral accessory lobe and then to the ilpc either only on the same side or bilaterally to the other antennal lobe as well. This latter route goes through an outer antenno-cerebral tract, and not the inner tract that is shown here used by the three projection interneuron fibers.

response that opened the doors for these earliest, precise studies of sex-pheromone receptor-neuron activity, correlating variations in neuronal responses with behavior (Kaissling 1974).

The advantages of using insect sex pheromone systems over vertebrate systems to study olfaction, touted in the early days by insect researchers and now borne out by the results, have been manifold. Insect pheromone systems illustrate the principles of odor quality blend discrimination with just two, and usually no more than four, odor components that account for the full-blown, powerful behavioral response (Baker 1989). Learning does not confound the reproducibility of the behavioral responses, which are reliably summoned up in discriminating behavioral assays, such as those involving upwind flight to the source of odor; other environmental influences are easily controlled experimentally, moreover, the sensitivities of insects to minute amounts of the correct blend are exquisite, as is their ability to discriminate among slight differences in blend ratios.

For the neurophysiological side of olfactory investigations, there are also advantages to using insect systems over vertebrate systems. The neuronal pathways that make up the olfactory system in insects are easily accessible, beginning with receptor neurons on the antennae that are housed in evaginated hairs that comb the air for odor molecules, instead of being imbedded in a sheet of mucus within an invaginated pocket—the nasal passages—in vertebrates. The neurons within receptor hairs can be conveniently studied by using tungsten electrodes to sample the activities of the one, two, or perhaps as many as four, neurons as they respond to the pheromone molecules. A second method for recording these neurons' activities is the cut-sensillum technique, which has now provided cutting-edge knowledge about the primary pathways of odor information into the antennal lobes of the insect brain. The antennal lobes are paired structures located at the base of the antennae that together make up the deutocerebrum, or the middle portion of the brain that is innervated by the antennae. The key advantage of the cut-sensillum technique is that it not only records the response profile of each antennal neuron to an array of odorants but also permits the staining of a physiologically identified neuron so that it can be followed to its termination in a particular region of the antennal lobe. The staining thereby provides a visual aid for determining how the information that a particular sensory

neuron carries may be transferred to the next layers of neurons in the antennal lobes, called interneurons.

The cut-sensillum technique was developed by Karl-Ernst Kaissling in Germany and first was used for studies of pheromone receptors on the antennae of the male silkworm, *Bombyx mori* (L.) (c.f., Kaissling 1974). Sharpened razors were used to remove the tip of a single antennal hair so that an electrical connection could be established with the receptor neurons. The method was modified by Van der Pers and den Otter (1978), who replaced the razors with sharpened glass micro-knives. The cut-sensillum technique is used in several European countries. We learned the technique in 1987 from Bill Hansson at the University of Lund in Sweden.

The cut-sensillum technique has been restricted mainly to recordings of sensory neurons within long trichoid hairs on insect antennae. Such hairs are the most accessible to cutting with the glass knives. Shorter basiconic hairs and other olfactory structures, such as pit pegs and plates, cannot be reached by the knives; thus, cuticular penetration with tungsten microelectrodes generally is a better method for recording from a wider variety of sensory structures. However, the cut-sensillum technique offers the advantage of being less sensitive to vibration and of allowing many hairs to be sampled within a very short time on the same antenna with a minimal effort. Another key advantage of this technique over tungsten recording is the ability to simultaneously monitor both the action potentials and direct current (DC) potentials of the neurons within the cut hair. DC-related potentials, considered by some to be generator potentials evoked solely from the neurons within the cut hair being monitored, cannot be recorded easily using tungsten because of the instability caused by unbuffered metal ions, and so interesting information about the receptor neurons' slow-waveform activities can be missed by using tungsten.

The results of neurophysiological studies of insect olfaction have indicated clearly that the first step in odor quality analysis involves the pheromone blend arriving on the antenna and being segregated immediately into its component parts through the selectivities of the different types of antennal neurons. The differential responsiveness to compounds is imparted by the peculiar receptor site proteins on the dendrites and, perhaps, even by the selectivities of binding proteins that bathe the neurons as a gel. The result is that the neurons respond op-

timally only to the molecules in the blend to which they are tuned, even though the complete blend of compounds has adhered to and even entered the hair and its binding protein milieux. The abundance of each pheromone component is, thus, first represented in the relative electrophysiological activities of the neurons specifically tuned to them. The action-potential activities of these thousands of neurons responding to their specific portion of the multicomponent blend then stream down without cross-communicating, in separate lines in the antennal nerve (the fiber tract comprised of the axons from all of the sensory neurons located in the antenna) to finally arrive at the macroglomerular complex (MGC) located at the base of both antennae in the antennal lobe. The MGC comprises varying numbers of concentrated neuropil called glomeruli that are made up primarily of axonal branches from antennal sensory neurons and the dendritic arborizations of interneurons that synapse with them. The MGC is found only in males and receives information exclusively from sex-pheromone sensitive antennal neurons. Antennal neurons that respond to plant- and flower-derived compounds terminate in what are termed ordinary glomeruli, located more ventrally in the antennal lobes of both males and females.
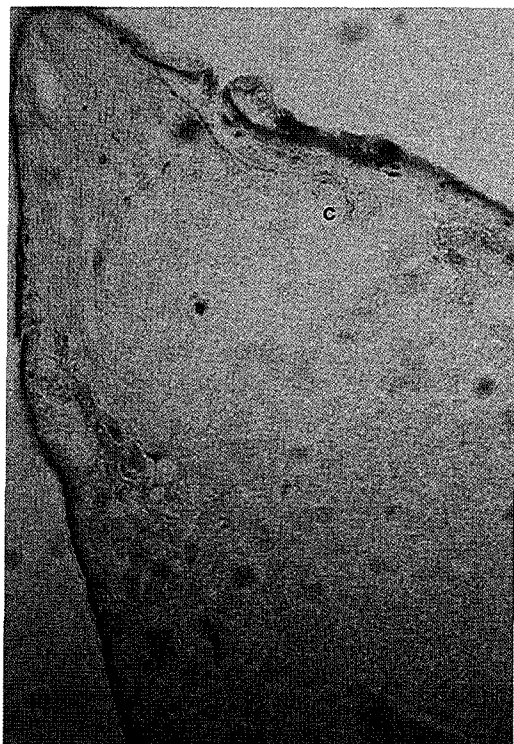
New insights into the morphology and function of the MGC were revealed by Bill Hansson and colleagues at the University of Lund who

Methylene blue-stained section of one antennal lobe from an adult male *T. ni*, showing the morphological compartmentalization of the macroglomerular complex into seven subcompartments (a–g), which sit like a cap on top of the more ventrally located ordinary glomeruli. Six of the macroglomerular subcompartments receive sex pheromone component-specific information from antennal neurons, and a seventh subcompartment (*c*) receives input about the alcohol (*Z*)-7-dodecenol (Z7-12:OH) that acts as a behavioral antagonist to upwind flight. The sex pheromone of the cabbage looper consists of a blend of six acetate esters (Ac). The major component, or most abundant component in the blend, is (*Z*)-7-dodecenyl acetate (Z7-12:Ac) ("Z" refers to the position of the hydrogens around the double bond in one of two geometrical isomers [the other is E] of the acetate), and neurons from the antenna tuned this component arborize in subcompartment *a*. The other five components are released in lesser quantities and are referred to as minor components: dodecenyl acetate (12:Ac), whose antennal neurons arborize in subcompartment *b*; (*Z*)-5-dodecenyl acetate (Z5-12:Ac), whose antennal neurons arborize in subcompartment *g*; 11-dodecenyl acetate (11-12:Ac), whose neurons terminate in subcompartment *e*; (*Z*)-7-tetradecenyl acetate (Z7-14:Ac), whose neurons terminate in subcompartment *d*; and (*Z*)-9-tetradecenyl acetate (Z9-14:Ac), whose neurons terminate in subcompartment *f*.

Whole mount of an antennal neuron from an adult male *T. ni* that responds specifically to the sex pheromone component (Z)-7-dodecenyl acetate (Z7-12:Ac). The neuron was stained with cobalt and intensified with silver to show its arborization within the middle of the macroglomerular complex. The brain is oriented such that lateral (the eye) is to the left of the photograph and medial (toward the other side of the head) is to the right. The antennal nerve enters the lobe from the top left of the photograph (reddish brown region).

Methylene-blue stained section of one antennal lobe of an adult male *T. ni* showing a (Z)-7-dodecenol (Z7-12:OH-sensitive) neuron stained with cobalt and arborizing in subcompartment *c* of the macroglomerular complex (MGC) The axon can be seen entering the MGC and traveling along the medial perimeter of the antennal lobe toward subcompartment *c*.

adapted the cut-sensillum technique, replacing the saline within the recording electrode with cobalt-lysine solution, and then inducing a single neuron within a sensillum to selectively take up cobalt dye only if it was stimulated for at least 1 hour by pulsed exposure to the pheromone component to which it was tuned. This procedure was first used on the adult moth *Agrotis segetum* Schiff (Hansson et al. 1992), and the results revealed in striking fashion that each glomerulus (referred to as subcompartments of the MGC) exclusively handles incoming information from the antenna about one specific component of the multicomponent pheromone blend. That there is an orderly and

reliable position of ordinary glomeruli in the insect brain from individual to individual was known from earlier anatomical work (Rospars 1983). However, it was the discoveries that there are MGC subcompartments and, more importantly, that they are functionally as well as spatially distinct that were breakthroughs for understanding animal olfactory processing (Hansson et al. 1991, 1992).

Being able to selectively stain functionally characterized sensory neurons is the final and, certainly, the singular advantage to using the cut-sensillum technique. We used this same staining procedure to trace the axonal projections of the cabbage looper, *Trichoplusia ni* (Hübner), to the MGC and showed that as in *A. segetum*, the sex pheromone component-specific neurons from *T. ni* male antennae arborize with high fidelity in their own MGC subcompartments (Todd et al. 1995). Over 383 attempts were made to stain a component-specific neuron with cobalt, but only 72 were successful, such that the pathways of the neurons could be traced to a glomerular subcompartment within the MGC. Some of these stained axons can be visualized in wholemount, but most are seen only by examination of serial sections of antennal lobe tissue. Even the axons from neurons tuned to the alcohol (Z)-7-dodecenol (Z7-12:OH) that functions as a behavioral antagonist to upwind flight progress target their own MGC subcompartment. The majority of our preparations contained only one stained neuron that in 98% of the preparations projected consistently to the same component-specific subcompartment. Although some double stainings also were obtained, these always involved the component-specific neuron arborizing with 100% fidelity in its primary location, with an additional neuron projecting to a second MGC subcompartment.
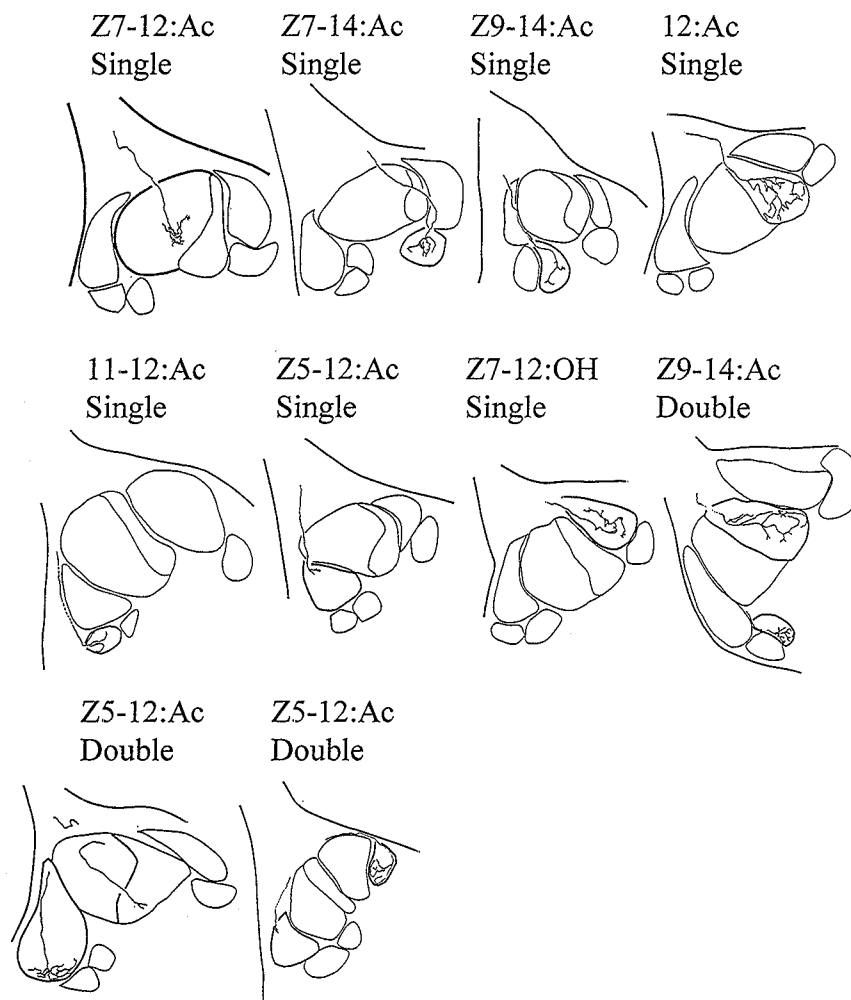
Within the antennal lobes, olfactory pathways of higher-order neurons, called local interneurons and projection neurons, can be monitored by using intracellular recording techniques and traced by using electrodes filled with dyes such as lucifer yellow (Anton and Hansson 1995). Again, in insects, these first integrative organs easily are accessible and can be penetrated with electrodes to sample the activities of the interneurons and monitor how they respond or do not respond to the activities of the ensemble of antennal neurons that is stimulated by the pheromone blend. Olfactory pathways can be further traced by staining interneurons still synaptically farther down the stream of information originating from the antenna, those in the protocerebrum and those

descending to the thoracic ganglia (Kanzaki et al. 1991a, b). Such neurons deep in the brain may have become "multimodal" through their confluence with the streams of visual information from the eyes.

Our work with *T. ni* (Todd et al. 1995), as well as research by the Hansson group, showed that the lines of pheromone component-specific information remain partitioned and are cleanly and spatially arranged at the bases of the antennae. Microscopic sections of silver-stained antennal lobes are now interpretable in new ways. They clearly show that as the antennal nerve with its large cable of thousands of axons enters the antennal lobe, it unravels into many smaller cables that must be component-specific lines that peel off and terminate in their particular component-specific MGC subcompartment.

For years, it has been understood by leaders in olfaction and taste research that odor quality discrimination must result from the ratios of neuronal activity being weighed across the many different types of receptor neurons, a phenomenon called "across-fiber patterning" (Dethier 1972). With recent advances, we can, however, see that the across-fiber pattern is now represented as an across-glomerular pattern as predicted by Rospars (1983): the pattern of glomeruli receiving input from the periphery defines the odor. With this understanding of the congruence of structure and function in MGC subcompartments, is it now easier to understand why perfumers and vintners can describe odors and flavors as having a particular "balance?" Is it now more understandable why flavors and fragrances can be described according to a spectrum of sound and as being particularly rich in bass, treble, or mid-range? We think so. We also think it is no surprise that the functional morphology of animal olfaction based on spatially arranged lines of component-specific information in glomeruli was first illustrated in insects (Hansson et al. 1991, 1992), given the long history of research in this field provided by entomologists.

Because of these neurophysiological and neuroanatomical breakthroughs, we now can begin to picture how the complete blend of odor in the pheromone is represented by a particular balanced array of neuronal activity across the glomerular subcompartments of the MGC. The array would be initially sampled by the first level of interneurons that reach into these pools of pure, component-specific activity, free of noise from other types of neurons and then weigh it against its neighbors for imbalance. These first interneurons, the "lo-

Z7-12:Ac Single   Z7-14:Ac Single   Z9-14:Ac Single   12:Ac Single

11-12:Ac Single   Z5-12:Ac Single   Z7-12:OH Single   Z9-14:Ac Double

Z5-12:Ac Double   Z5-12:Ac Double

Serial reconstructions of antennal neurons from adult male *T. ni* that were stained with cobalt and intensified with silver, showing their projections in specific subcompartments of the macroglomerular complex (MGC). The pathways and destinations of axons in the antennal lobe were drawn using a camera lucida, which projected the images of successive 10-μm antennal lobe sections stained with methylene-blue to highlight the glomeruli. The images of both the tissue sections and the drawings of them were viewed in a compound stereo microscope and the black-stained neuron traced through successive sections. Even though the number of successful stainings was small, the propensity for each component-specific neuron to send its axon into a particular glomerular subcompartment was highly consistent (Todd et al. 1995). In single stainings resulting from stimulation by any one of the six acetate (Ac) sex pheromone components (38), there was 100% fidelity of arborization to a particular glomerular subcompartment. Our neurophysiological and neuroanatomical investigations thus showed that when the complete six-component sex pheromone released by a female *T. ni* contacts the male's antennae, component-specific activity from the six different acetates will travel into six different subcompartments of the male's MGC neuropil. This is the blend that evokes optimal mate-finding behavior at all phases of the response, from initiation of flight to hairpencil display (close-range precopulatory behavior). Neurons specific for the behavioral anatagonist alcohol (OH) Z7-12:OH also project into a particular glomerular subcompartment. Reconstructions of some of the doubly stained preparations indicate that behavioral redundancy can occur when a single compound stimulates two different types of receptor neurons. Two glomerular destinations in the MGC can then be stimulated by one component and, thus, the complete ensemble of glomerular activity between the six types of antennal neurons and the local interneurons that sample the activity can remain unaffected by one or two missing components. We suggest that the degree of cobalt uptake mirrors the relative affinities of the stained neurons' dendritic receptor sites for the stimulus component, and the propensity of a second neuron to stain in addition to the primary component-targeted neuron would be indicative of its propensity to fire (generate action potentials) in response to that component. For this cross-stimulation to translate into behavior, it must ultimately manifest itself in the relative frequency of action potentials generated by the neurons involved.

Frontal section of one antennal lobe of an adult male *T. ni* stained and intensified with silver, showing the axons entering the macroglomerular complex from the antennal nerve arranged as smaller fascicles that peel away and target their respective glomerular subcompartments (a–g). Ax, axon: L, lateral; M, medial; Ord, ordinary glomeruli.
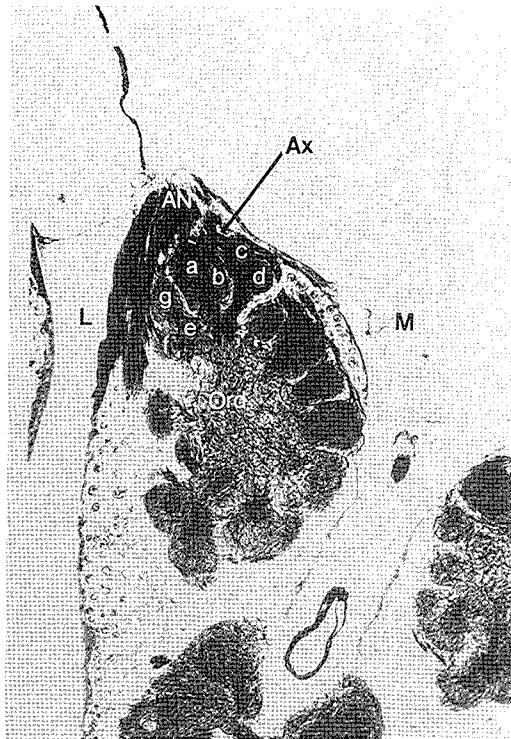
cal" interneurons, spread across the antennal lobe like a net, never traveling out of the antennal lobe, and interconnect the glomerular subcompartments. Their weighing ability is indicated by the fact that for many of them, their neurotransmitter is gamma aminobutyric acid (GABA), which imparts inhibitory activity to the receiving neurons. Therefore, a greater acetylcholinergic excitation of a local interneuron by antennal neuronal synapses occurring in one component-specific glomerulus would produce a greater amount of GABA-ergic inhibition that spreads to the other subcompartments. Imbalance in the blend might thus be enhanced by the lateral inhibition imparted by these local interneurons, just as any edges between light and dark in a visual field are enhanced by the lateral inhibition imparted by amacrine cells in the initial levels of the optical pathways.

In the adult male moth, pheromone blend information leaves the MGC and travels to locations deeper in the brain through a very small number of interneurons. These are called projection interneurons because they project out of the MGC to higher brain centers. Unlike local interneurons, projection interneurons usually arborize only in a limited number of subcompartments in the MGC and generally travel to the far back of the brain where they synapse with neurons in the calyx of the mushroom body before continuing on to synapse with other neurons in the extreme lateral portion of the protocerebrum.

There are many types of integration that can be performed by local and projection interneurons that enable them to take the single-component information provided from the sensory neurons and produce the sensation of a blend. In seemingly counterproductive fashion, by the time pheromone information has reached the local interneurons, the orderly spatial arrangement of odor-component lines preserved in MGC glomerular subcompartments has been reshuffled and blended (Anton and Hansson 1995). Contributing to this rearrangement is not only lateral inhibition from the local interneurons but also disinhibition. Evidence for the latter comes from simultaneous recordings from local and projection interneurons in the adult tobacco hornworn, *Manduca sexta* (L.), by Tom Christensen and coworkers (Christensen et al. 1993). Also, it is clear from results from *Spodoptera littoralis* (Boisduval), as well as *A. segetum*, that often a projection interneuron will arborize in one particular MGC subcompartment yet it will relay information about a sex pheromone component known to be coming into the MGC from antennal neurons arborizing in a completely different MGC subcompartment (Anton and Hansson 1995). The reshuffling of labelled-line information during odor quality integration in the antennal lobe also is evident in that other projection neurons have been found that are equally responsive to two or three different single components, yet they arborize in only one component-specific MGC subcompartment.

Recent findings with *A. segetum* have shown that the first level at which blends are uniquely responded to by neuronal elements is at the local interneuron level. But not to be overlooked is that a major amount of the information traveling to the protocerebrum via projection interneurons involves not blends, but information about the pure individual components, because there are many projection interneurons that respond only when a particular individual component is present, regardless of whether or not it is part of a blend. This does not mean that the sensation of blend also cannot be shaped by protocerebral interneurons, or even descending interneurons that could weigh the ratios of electrophysiological activity of component-specific projection interneurons. It has been shown already that at the local interneuron level, there are some neurons that do discriminate between one blend and another. In fact, some local interneurons in males from the Swedish race of *A. segetum* respond preferentially to the blend emitted by Swedish

females and not to the blend from Zimbabwean females (Wu et al. 1996). There are also several examples of blend-sensitive projection interneurons that respond only if a blend is present and not to any individual component. Because local interneurons are now known to be capable of responding uniquely to blends, we cannot rule out the possibility that blend-sensitive projection interneurons may merely be relaying a blend-integrating response that originated in local interneurons.

Regardless, there are several streams of information about the pheromone blend that emerge from the antennal lobe and travel to centers deeper in the brain along various functionally different types of projection interneurons. It cannot be ruled out that there are also synaptic connections directly between antennal neurons and projection interneurons without local interneurons being involved, but recordings from adult *M. sexta* indicate that this is not the case for this species. There is evidence that inhibitory local interneurons can synapse with each other to create excitation in projection interneurons through disinhibition (Christensen et al. 1993). There is certainly much more to learn about all of the ways in which pheromone blend quality integration is performed in the MGC of male moth antennal lobes.

Blend-integrating responses carried by projection interneurons are known to be of at least two different types. One type involves a long-lasting excitation that occurs in response only to the presence of a blend of two components on the antenna and not to each component individually. This type of response is characterized as being the typical blend-integrating response, and such a response has been discovered in antennal lobe interneurons of the tobacco budworm, *Heliothis virescens* (F.), and the corn earworm, *Helicoverpa zea* (Boddie) (Christensen et al. 1991). However, another important type of blend-integrating response also is known from *M. sexta*, in which the presence of the blend on the antenna sharpens the onset and offset of the train of spikes emerging from these interneurons, resulting in a more highly phasic burst than would otherwise occur in response to either component alone. The temporal sharpening occurs due to a combination of inhibition from neuronal elements responding to one of the two pheromone components and excitation from those elements responding to the other. Thus, two different types of blend-specific information, based on the temporal aspects of the response—either more long-lasting and tonic or phasic—travel

out through the projection interneurons to the brain. Baker (1990) has suggested that the long-lasting response sustains casting flight in pockets of clean air between filaments, and the phasic response permits repeated upwind surges in response to the filaments themselves.

Morphologically, there appear to be at least two projection interneuron pathways, one long and the other short, from the antennal lobe into the protocerebrum. The long route might be considered to be the more "cerebral" because it involves the mushroom bodies and lateral protocerebrum. This pathway, one on each side of the brain, originates in each antennal lobe, travels out to the very back of the brain, and arborizes in the calyx of the mushroom body before continuing out to the side of the brain to arborize in the lateral protocerebrum. Most projection interneurons have been shown to send information out this way, but there are some interneurons that project to the inferior lateral protocerebrum.

In contrast, a shorter, perhaps more reflexive, pathway was first indicated in the results of Kanzaki et al. (1991b) on adult *M. sexta*, who found protocerebral interneurons and descending interneurons that seemed to take information from the antennal lobe by way of another synaptic area, the lateral accessory lobe, and send it more directly down to the flight motor in the subesophageal and thoracic ganglia. As mentioned earlier, controlled flight upwind to the female cannot occur without visual flow-field information (direction and speed of movement of ground patterns across the eyes), which indicates wind direction and determines upwind progress. Therefore, for either the long or the short pathways to be involved in upwind flight to the source, they would need to have input from motion detectors in the eyes blended into them at some point during their descent to flight motor systems. Such confluence of visual motion and pheromone responsiveness could, in fact, occur in the lateral accessory lobe, and multimodal visual and olfactory descending interneurons have been shown to occur in the gypsy moth, *Lymantria dispar* (L.) (Olberg and Willis 1990).

By applying "cutting-edge" techniques, researchers in the field of insect pheromone olfaction are making progress toward understanding the mechanisms that result in the discrimination of particular odor blends and their subsequent behavioral effects. These mechanisms rely very heavily on the preservation of odor-component-specific information in antennal lobe glomeruli, followed by exten-

sive sampling and integration of these lines by both local and outgoing antennal lobe interneurons. The integrated results of behavioral, neurophysiological, and neuroanatomical studies such as we have outlined here may aid entomologists in developing more effective olfactory signals. The effectiveness of these signals depends ultimately on our knowledge of olfaction and how intelligently we can manipulate olfactory pathways to prevent mate-finding, oviposition, and other odor-mediated behaviors.

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