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It is an extraordinary and undeserved honor to present the fourth Jan de Wilde Memorial Lecture. Although I did not have the privilege of personal acquaintance with Jan de Wilde, I have admired his work and his high standing in science ever since I decided to commit myself to the study of insect chemical senses. It is inspiring to learn about his parallel commitments to scientific discovery, education, international collegiality, and the enhancement of human welfare through scientific research. I hope that Professor de Wilde would have found the work outlined here both interesting and promising.

Olfaction in insects

In insects, olfaction plays a major role in the control of many kinds of behavior. Orientation and movement toward, and interactions with, receptive mating partners, appropriate sites for oviposition, sources of food, and hosts for parasitism usually involve olfactory signals that initiate, sustain, and guide the behaviors. Because of their prominence in the zoosphere, their agricultural and medical importance, and their usefulness as models for both behavioral and neurobiological research, insects have been extensively studied by investigators interested in mechanisms of olfactory control of behavior. These creatures respond to a variety of semiochemicals, including pheromones (chemical messengers within a species, such as sex attractants) and kairomones (chemical messengers between species and adaptively favorable to the recipient, such as attractants and stimulants for oviposition and feeding emitted by a host plant). Studies of the responses of insects to such biologically significant odors have shown that the quality and quantity of odorants in complex mixtures present in the environment are encoded in patterns of activity in multiple olfactory receptor cells in the antennae. These "messages" are decoded and integrated in the olfactory centers of the central nervous system (CNS), and it is there that olfactorily induced changes in the behavior or physiology of the insect are initiated.

Of paramount interest, both historically and currently, is the attraction of a mating partner by means of a chemical signal -- the sex pheromone -- released by a receptive individual of one sex and detected by conspecifics of the opposite sex. In moths, these chemical signals are the primary means by which females broadcast their sexual receptiveness over relatively long distances to conspecific males. The male moths respond to the sex-pheromonal stimulus with well-characterized mate-seeking behavior involving arousal, patterned anemotactic flight, short-range orientation to the calling female, and mating[1].

Behaviorally relevant olfactory mechanisms have been probed in depth in several insect taxa, including cockroaches[2], honey bees[3,4], and fruit flies[5], but the most extensive explorations of the detection and processing of, and behavioral responses to, sex pheromones have been carried out in moths. Building upon earlier and recent work of others[6-11] and paralleling current research using different insect species, my coworkers and I study the olfactory system of the experimentally favorable giant sphinx moth *Manduca sexta*. We have investigated this model olfactory system extensively by means of anatomical, neurophysiological, biochemical, cytochemical, developmental, and cell-culture methods. This brief review focuses on some of our findings and speculations about the functional organization and physiology of the olfactory system in *M. sexta*; more detailed reviews of this and other aspects of our work have been presented elsewhere[12-15].

A principal long-term goal of our research is to understand the neurobiological mechanisms through which information about a specific olfactory signal, the female's sex pheromone, is detected and integrated with inputs of other modalities in the male moth's brain and how the message ultimately initiates and controls the characteristic behavioral response of a conspecific male moth. Pursuit of that goal promises to teach us much about how the brain processes chemosensory information and uses it to shape behavior. Our studies along this line to date have persuaded us that the male's olfactory system consists of two parallel sub-systems, one a sexually dimorphic "labelled-line" pathway specialized to detect and process information about the sex pheromone, and the other a more complex pathway that processes information about plant (and probably other environmental) odors encoded in "across-fiber" patterns of physiological activity.

The sex-pheromonal stimulus

The sex pheromones of moths generally are mixtures of two or more chemical components, typically aldehydes, acetates, alcohols, or hydrocarbons produced in specialized glands by metabolism of fatty acids[1]. Often, the species-specific blend of components is the message, and males of many moth species, including *M. sexta*, give their characteristic, qualitatively and quantitatively normal behavioral responses only when stimulated by the correct blend of sex-pheromone components and not to individual components or partial blends lacking key components[16,17].

Solvent washes of the pheromone gland of female *M. sexta* yield 12 substances: 8 C_{16} and 4 C_{18} aldehydes[17]. A synthetic mixture of these components elicits the same behavioral responses in males as does the signal released by a calling female[17,18]. A blend of 2 of the components, the dienal (*E*,*Z*)-10,12-hexadecadienal (*E*10,*Z*12-16:AL or bombykal[18]) and the trienal (*E*,*E*,*Z*)-10,12,14-hexadecatrienal (*E*10,*E*12,*Z*14-16:AL), elicits the normal sequence of male behaviors in a wind tunnel, but the individual components are ineffective[17]. Field-trapping studies show that a blend of all 8 of the C_{16} aldehydes (including the *E*,*E*-isomer of bombykal and the *E*,*E*,*E*-isomer of the trienal) present in the gland washes is significantly more effective in attracting males than are blends of fewer components, suggesting that all of the C_{16} aldehydes have a role in the communication system of *M. sexta* -- i.e. that the sex pheromone of this species comprises those 8 C_{16} aldehydes (ref. 17 and Tumlinson, personal communication). Our neurophysiological studies have focused on 3 important properties of the sex-pheromonal signal: its quality (chemical composition of the blend), quantity (concentrations of components), and intermittency (owing to the fact that the pheromone in the plume downwind from the source exists in filaments and blobs of odor-bearing air interspersed with clean air[19,20]). Each of these properties of the pheromonal message is important, as the male moth gives his characteristic behavioral responses only when the necessary and sufficient pheromone components are present in the blend[17] (qualitative requirement), when the concentrations and blend proportions of the components fall within acceptable ranges[21] (quantitative requirement), and when the pheromone blend stimulates his antennae discontinuously[9,22] (intermittency requirement). We examine how each of these important aspects of the odor stimulus affects the activity of neurons at various levels in the olfactory pathway.

Detection of the sex pheromone

Each antenna of adult *M. sexta* comprises two basal segments (scape and pedicel), containing mechanosensory organs, and a long sexually dimorphic flagellum divided into ca. 80 annuli bearing numerous sensilla of several types, the great majority of which are olfactory[23,24]. Antennal flagella of both male and female *M. sexta* (whose larvae feed, and adult females oviposit, exclusively on plants of the family *Solanaceae*) have many olfactory receptor cells that respond to volatiles given off by plants[25] and presumably are involved in host-plant recognition and discrimination. In addition, the antennae of the male moth possess receptor cells specialized to detect individual components of the female's sex pheromone[25,26].

A male flagellum has ca. 3×10^5 sensory neurons associated with ca. 10^5 recognized sensilla, of which ca. 40% are male-specific sensory hairs or *sensilla trichodea*[23,25,26-28]. Type-I trichoid hairs ($\leq$ 600 μm long) are typical olfactory sensilla, having single walls with pores, and are innervated by two olfactory receptor cells that send their unbranched dendrites through the lumina of the fluid-filled hairs to their tips[23,27-29]. One of these two male-specific receptor cells is highly sensitive and specific to *E*10,*Z*12-16:AL, while the second receptor cell is "tuned" to *E*10,*E*12,*Z*14-16:AL in about 85% of these sensilla or to *E*10,*E*12,*E*14-16:AL in the remaining 15% of the sensilla[26]. Pheromone-specific receptor cells in moth antennae thus represent information about stimulus quality by means of their specialization as narrowly tuned input channels. Groups of these cells can "follow" intermittent pheromonal stimuli at naturally occurring frequencies (up to ca. 10 pulses per sec)[30].

Functional organization of central olfactory pathways

Axons of antennal receptor cells project through the antennal nerve to enter the brain at the level of the ipsilateral antennal lobe (AL) of the deutocerebrum[24]. Olfactory receptor cell axons and possibly also fibers of other modalities (such as hygroreceptors) project from the flagellum to targets in the AL, but axons of mechanosensory neurons in the basal segments of the antenna bypass the AL and project instead to a deutocerebral "antennal mechanosensory and motor center" posteroventral (with respect to the body axis of the animal) to the AL[24,30,31]. In moths and some other insect taxa, sex-pheromonal information is processed in a prominent male-specific area of neuropil in each AL called the macroglomerular complex (MGC)[24,31,32].

The AL has a central zone of coarse neuropil (largely neurites of AL neurons) surrounded by an orderly array of glomeruli, including 64 ± 1 spheroidal "ordinary" glomeruli and, in the male, the sexually dimorphic MGC near the entrance of the antennal nerve into the AL[31,33]. Bordering this neuropil are 3 groups of AL neuronal somata -- lateral, medial, and anterior -- totalling about 1200 cells[12,32,34]. Each olfactory axon projecting from the antennal flagellum into the brain terminates within a single glomerulus in the ipsilateral AL[31,35], where it makes chemical synapses with dendritic processes of AL neurons[32,35,36]. These inputs are believed to be cholinergic[37] and the postsynaptic elements, to be largely or exclusively LNs[36]. The ordinary glomeruli, which are condensed neuropil structures 50-100 μm in diameter, contain terminals of sensory axons, dendritic arborizations of AL neurons, and all of the primary-afferent synapses and synaptic connections among AL neurons, and they are neatly surrounded by an investment of glial processes[32,35,38].

With very few exceptions, the neurons in the medial, lateral, and anterior cell groups of the AL fall into two main classes[12,32,34,39,40]. Projection neurons (PNs or output neurons) have dendritic arborizations in the AL neuropil and axons that project out of the AL, and local interneurons (LNs) lack axons and have more or less extensive arborizations confined to the AL neuropil. The PNs relay information about odors, synaptically processed and integrated in the AL by neural circuitry involving sensory axons, LNs, and PNs, to higher-order centers in the brain[34]. Many PNs have dendritic arborizations confined to single AL glomeruli and axons that project via the inner antenno-cerebral tract through the ipsilateral protocerebrum, sending branches into the calyces of the ipsilateral mushroom body and terminating in characteristic olfactory foci in the lateral protocerebrum. Other PNs have arborizations in one or more AL glomeruli and send axons via the middle, outer, dorsal, and dorso-medial antenno-cerebral tracts to characteristic regions of the protocerebrum, including lateral and inferior regions[12,34].

Axons of male-specific antennal olfactory receptor cells specialized to detect components of the species-specific sex pheromone project exclusively to the MGC[31,41], and all AL neurons that respond to antennal stimulation with sex-pheromone components have arborizations in the MGC[32,39,40]. The MGC in *M. sexta* has two major, easily distinguishable divisions: a doughnut-shaped neuropil structure (the "toroid") and a globular structure (the "cumulus") adjacent to the toroid and closer to the entrance of the antennal nerve into the AL[41]. AL PNs that respond to antennal stimulation with *E*10,*Z*12-16:AL have arborizations in the toroid and PNs responsive to *E*10,*E*12,*Z*14-16:AL, in the cumulus[41]. Thus first-order synaptic processing of sensory information about these important sex-pheromone components, which are necessary and sufficient to elicit and sustain the male's behavioral response, apparently is confined to different, distinctive neuropil regions of the MGC.

Processing of sex-pheromonal information in the male antennal lobe

In our studies of central processing of sex-pheromonal information in *M. sexta*, we again focus on the qualitative, quantitative, and intermittency properties of the pheromonal signal. We examine how each of these aspects of the pheromonal stimulus affects the activity of the two main classes of AL neurons associated with the MGC: the male-specific local and projection neurons.

Stimulus quality. By means of intracellular recording and staining methods, we have examined the activity of AL neurons in response to stimulation of the ipsilateral antenna with each of the sex-pheromone components as well as partial and complete blends[42]. In accordance with results of behavioral and sensory-receptor studies, we have found that *E*10,*Z*12-16:AL and *E*10,*E*12,*Z*14-16:AL are the most effective and potent sex-pheromonal components for eliciting physiological responses in the AL neurons. On the basis of these responses, we classified the neurons into two broad categories: pheromone generalists and pheromone specialists[43]. Pheromone generalists are neurons that respond similarly to stimulation of either the *E*10,*Z*12-16:AL input channel or the *E*10,*E*12,*Z*14-16:AL input channel and do not respond differently when the complete, natural blend is presented to the antenna. These cells may therefore be involved in mediating general arousal in response to sex pheromone but apparently do not contribute to species recognition. In contrast, we refer to neurons that can discriminate between antennal stimulation with *E*10,*Z*12-16:AL from stimulation with *E*10,*E*12,*Z*14-16:AL as pheromone specialists. There are several types of pheromone specialists. Some receive input only from the dienal channel or the trienal channel. These cells therefore preserve information about individual components of the species-specific blend. Similar specialist responses have been reported even for higher-order neurons descending from the brain in the silkmoth *Bombyx mori*[44]. These observations suggest that information about specific components of the blend, and not only the complete blend, is relevant to chemical communication in these animals.

An important subset of pheromone-specialist PNs found in male *M. sexta* receives input from both *E*10,*Z*12-16:AL and *E*10,*E*12,*Z*14-16:AL channels, but the physiological effects of the two inputs are opposite[39]. That is, if antennal stimulation with the dienal leads to excitation, then stimulation with the trienal inhibits the interneuron, and vice versa. Simultaneous stimulation of the antenna with both *E*10,*Z*12-16:AL and *E*10,*E*12,*Z*14-16:AL elicits a mixed inhibitory and excitatory response in these special PNs. Thus these neurons can discriminate between the two inputs based upon how each affects the spiking activity of the cell. These cells also respond characteristically to the natural pheromone blend released by the female: pheromone-specialist neurons of this type have unmatched ability to follow intermittent pheromonal stimuli occurring at natural frequencies (up to 10 pulses per sec)[45] (see below).

While most of the olfactory interneurons encountered in male *M. sexta* ALs respond preferentially to *E*10,*Z*12-16:AL and *E*10,*E*12,*Z*14-16:AL, some neurons also respond to other C_{16} components in the female's pheromone blend, including the isomeric trienal *E*10,*E*12,*E*14-16:AL[41]. Taken together with the aforementioned field-trapping evidence that all of the C_{16} components in the solvent washes of female sex-pheromone glands have behavioral effects, these findings suggest that "minor" components of the blend may play subtle but important roles in pheromonal communication. In addition, sex-pheromonal components from another species can sometimes stimulate olfactory receptor cells in a male moth's antennae and thereby affect his behavior, as is the case of receptor cells in the antennae of male *Helicoverpa zea*[43].

Stimulus Quantity. Numerous studies in the field and in wind tunnels have shown that pheromone-mediated orientation is a dose-dependent phenomenon[46]. We therefore have examined the ability of AL neurons to encode changes in the concentration of a pheromonal stimulus[43]. When a male's antenna is stimulated with a series of pheromonal stimuli of graded concentrations, MGC PNs exhibit various dose-response relationships. In some of these PNs, the dynamic range of the cell extends up to the highest concentration tested (0.1 female equivalents (FE) of sex pheromone), but in other MGC PNs, inactivation of spiking occurs between 0.01 and 0.05 FE. Some PNs that have this ability to encode quantitative information about the pheromone yield a dose-response relationship, as measured by number of spikes elicited, that is quite linear up to 0.05 FE but falls off above this concentration. The response as measured by maximum instantaneous frequency of spiking, however, continues to increase up to the maximum concentration tested (0.1 FE). A corresponding increase in the amplitude of the membrane depolarization can also be seen. In contrast, some other concentration-sensitive PNs give a spiking response that falls off between 0.01 and 0.05 FE. Membrane depolarization continues to increase with concentration, suggesting that attenuation and gradual disappearance of the spikes are due to sodium inactivation at these high levels of membrane depolarization.

Stimulus intermittency. One of the most important characteristics of a female moth's sex-pheromone plume is its nonuniformity. Simulations of odor plumes using ionized air have shown clearly that a plume is not a simple concentration gradient but instead possesses a distinctly discontinuous and filamentous structure[19,20]. Furthermore, abundant behavioral evidence shows that a male moth's ability to locate a pheromone source is greatly improved if the odor plume is discontinuous[46]. Because spatial discontinuity of the pheromonal signal in the environment is detected by a flying male moth as temporally intermittent stimuli, we have sought to learn how intermittent pheromonal stimuli delivered to a male's antenna are registered by MGC PNs in his ALs. We discovered that certain pheromone-specialist PNs are especially capable of following rapidly pulsed pheromonal stimuli with corresponding bursts of impulses. These are the cells cited above that can discriminate between *E*10,*Z*12-16:AL and *E*10,*E*12,*Z*14-16:AL because one of these key components excites the cell while the other inhibits it. This inhibitory input to such PNs enhances their ability to follow brief pulses of pheromone blends delivered at frequencies up to 10 pulses per sec by controlling the duration of excitatory responses and preparing the PN for the next bout of excitation[45].

Higher-order processing of sex-pheromonal information in the central nervous system

After synaptic processing in the AL, information about sex pheromone and other odors is relayed to higher centers in the protocerebrum by way of the axons of AL PNs. Toward the goal of understanding how pheromonal information controls the behavior of male moths, we have begun to explore the physiological and morphological properties of neurons in the protocerebrum that respond to stimulation of the antennae with sex pheromone or its components[47,48].

We find that many pheromone-responsive protocerebral neurons have arborizations in a particular neuropil region, the lateral accessory lobe (LAL), which appears to be important for processing of olfactory information[47]. The LALs are situated lateral to the central body on each side of the protocerebrum. Each LAL is linked by neurons that innervate it to the ipsilateral superior protocerebrum as well as the lateral protocerebrum, where axons of AL PNs terminate[33,39,40]. The LALs are also linked to each other by bilateral neurons with arborizations in each LAL. Neuropil adjacent to the LAL contains branches of many neurons that descend in the ventral nerve cord. Local neurons link the LAL to this adjacent neuropil. Some descending neurons also have arborizations in the LAL. Thus, the LAL is interposed in the pathway of olfactory information flow from the AL through the lateral protocerebrum to descending neurons.

All protocerebral neurons observed to date to respond to antennal stimulation with pheromone were excited.

Although brief IPSPs were sometimes elicited in mixed inhibitory/excitatory responses, sustained inhibition was not observed. Certain protocerebral neurons show long-lasting excitation (LLE) that sometimes outlasts the olfactory stimuli by up to 30 sec. In some other protocerebral neurons, pheromonal stimuli elicit brief excitations that recover to background firing rates < 1 sec after stimulation. LLE is more frequently elicited by the sex-pheromone blend than by *E*10,*Z*12-16:AL or *E*10,*E*12,*Z*14-16:AL. LLE responses to pheromonal stimuli were observed in more than 50% of the bilateral protocerebral neurons sampled that had arborizations in the LALs. Fewer than 10% of the protocerebral local neurons examined exhibited LLE in response to similar stimuli. AL PNs responding to pheromone components do not show LLE[39,40]. Thus, LLE appears not to be produced at early stages of olfactory processing in the AL, but first occurs at the level of the protocerebrum.

These findings suggest that the LAL is an important region of convergence of olfactory neurons from other regions of the protocerebrum. Synaptic interactions in the LAL may mediate integration of both ipsilateral and bilateral olfactory information prior to its transmission to the bilateral pool of descending neurons. LLE appears to be one important kind of physiological response that is transmitted to thoracic motor centers. How this LLE might participate in the generation of the male moth's characteristic behavioral response is a subject of ongoing research.

Concluding remarks

Relatively little is known about central processing of olfactory information about plant volatiles in the brains of moths and butterflies. These important substances include odorants that influence feeding and oviposition behaviors. It is likely that information about plant odors is integrated in the CNS with that from other odor sources in the environment. Studies to date in *M. sexta* have shown that the non-sexually-dimorphic AL neurons, and the ordinary glomeruli in which they have their dendritic arborizations, are the sites of initial processing of plant odors. Some pheromone-responsive, sexually dimorphic LNs in the AL also respond to antennal stimulation with plant odors, but because male-specific PNs are only weakly inhibited by, or unresponsive to, plant odors, the significance of the findings in LNs is unclear. Further insights into the functions of the lepidopteran olfactory system will depend upon successful unravelling of the mechanisms involved in processing and integrating information from pheromonal, plant, and other biologically important odors in the CNS. Moreover, the reproductive success of many insects depends upon the integration of processed olfactory information with input from other sensory modalities. These integrative mechanisms, which ultimately lead to the production or modulation of coordinated and stereotyped motor outputs and thereby to characteristic patterns of behavior, are among the most interesting problems in contemporary insect-neurobiological research.

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