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Analysis of chemical signals by nervous systems

(olfaction/glomeruli/pheromone/chemoreception/insect)

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ABSTRACT **Intraspecific and interspecific communication and recognition depend on olfaction in widely diverse species of animals. Olfaction, an ancient sensory modality, is based on principles of neural organization and function that appear to be remarkably similar throughout the zoosphere. Thus, the "primitives" of olfactory stimuli that determine the input information of olfaction, the kinds of "molecular images" formed at various levels in the olfactory pathway, and the cellular mechanisms that underlie olfactory information processing are comparable in invertebrates and vertebrates alike. A case in point is the male-specific olfactory subsystem in moths, which is specialized to detect and analyze the qualitative, quantitative, and temporal features of the conspecific females' sex-pheromonal chemical signal. This olfactory subsystem can be viewed, and is here presented, as a model in which common principles of organization and function of olfactory systems in general are exaggerated to serve the requirements of a chemical communication system that is crucial for reproductive success.**

All creatures detect and react to chemicals in the external environment. In metazoans possessing a differentiated nervous system, an important function of that system is to detect, analyze, integrate, and generate responses to chemicals in the environment. Among the substances to which these organisms must respond are chemical signals, including pheromones and kairomones. Pheromones are chemical messengers between individuals of the same species, such as the sex attractants of moths and the alarm pheromone of honey bees. Kairomones are chemical messengers between species and adaptively favorable to the recipient, such as attractants and stimulants for insect oviposition and feeding emitted by a host plant. The importance of such chemical signals for survival and reproductive success is reflected in remarkable chemosensory capacities and specializations in diverse species of animals.

After considering the evolutionary origins of the olfactory system and some basic principles of olfaction, this brief review examines one of the most extensively studied examples of neural processing of semiochemical information: the sex pheromone-specific olfactory subsystem in male moths. This male-specific subsystem can be viewed as representing an exaggeration of organizational principles and functional mechanisms that are characteristic of olfactory systems in general.

Origins of Olfactory Systems

Consideration of the origins and evolution of chemosensation can help one to begin to understand the themes of olfaction and chemical communication that are common to diverse phyletic groups. This section emphasizes ideas that were propounded with characteristic clarity and elegance by the late Vincent Dethier in his 1990 R. H. Wright Lectures on Olfaction. Because the published version of those lectures (1) may not be widely accessible, some of Dethier's main points are restated here.

Origins of Chemoreception. The universal chemoreceptive capacity of living organisms surely must have arisen in the earliest cells, at the dawn of life billions of years ago (1). That capacity enables a cell to respond to substances without the necessity of internalizing or metabolizing them and is fundamental to the living state.

Studies of unicellular organisms have afforded insights about the origins of chemosensory processes and mechanisms exhibited by metazoa. Thus, modern bacteria such as *Escherichia coli* (2, 3) sense and respond to chemicals in ways that probably resemble those of ancient prokaryotes. *E. coli* possess finely "tuned" receptors for specific substances in the environment, mechanisms for transducing the stimuli and for decoding, integrating, and transmitting information about them, and means to generate appropriate behavioral responses. The motifs of chemoreception are conserved and elaborated in the protists and especially the slime molds (1, 4), suggesting subsequent evolutionary transitions. Dethier observed (1):

> With the advent of multicellularity many cells lost some of their ancient skills, but the organism's capability of sensing the chemical richness of the world was not impaired. Chemoreception became the prime function of specialized strategically situated cells anchored in epithelial sheets. The coupling of chemoreception to motility, that is, to behavioral responses, was accomplished by close association with transmitting systems. Transitional stages between the two functional levels, self-contained unicellular systems and neurally-linked multicellular systems, are preserved in contemporary coelenterates. Here are to be found the earliest metazoan chemoreceptors. In the further evolution of the nervous system there was a division of labor associated with a diversification of kinds of neurons, segregation in which like units gathered together, and compartmentalization of functional assemblies within ganglia.

Whence Olfaction? Evolution of metazoan chemoreception eventually gave rise to anatomically and functionally distinct chemosensory systems—olfactory and gustatory—which are distinguishable, in organisms that have a central nervous system (CNS), on the basis of the disposition of chemoreceptor cells and the central organization of their afferent axons (1).

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Abbreviations: AL, antennal lobe; CNS, central nervous system; FE, female equivalent; GABA, γ-aminobutyric acid; IPSP, inhibitory postsynaptic potential; LAL, lateral accessory lobe; LLE, long-lasting excitation; LN, local interneuron; MGC, macroglomerular complex; ORC, olfactory receptor cell; PN, projection neuron.

Whereas both olfaction and taste are served by receptor cells densely arrayed in epithelia and exposed to the environment, the typifying feature of an olfactory system is the projection of axons of olfactory receptor cells (ORCs) to discrete, condensed synaptic glomeruli in the CNS. The difference between the central organization of projections of gustatory receptors and ORCs reflects basic functional differences between these two chemical senses: the numbers of receptor cells, substances that normally stimulate those receptors, and qualities or categories of stimuli that can be discriminated are smaller for taste than for olfaction.

Olfactory glomeruli must have evolved early, because these characteristic structures are present in the "olfactory brains" of modern representatives of ancient marine groups including molluscs (5) and crustaceans (6). Likewise the lampreys, which are extant representatives of the most primitive vertebrates, have relatively large olfactory bulbs with glomeruli and conspicuous mitral cells not unlike those of more advanced vertebrates (7).

As animals emerged from the seas to inhabit the land, chemosensory systems had to adapt to terrestrial conditions. In particular, the olfactory system had to detect sparse molecules of diverse volatile substances in the desiccating ambient atmosphere. In both vertebrates and invertebrates, olfactory organs of marine forms became adapted for smelling on land. For example, antennae, which had appeared in many classes of marine arthropods starting as early as the Cambrian period and can be assumed (on the basis of knowledge about contemporary crustaceans) to have served an "olfactory" function, were brought along—with appropriate modifications—by animals that made the transition to the land. Contemporary representatives of the phylum Onychophora, terrestrial animals that have similarities to both annelids and arthropods and have changed little since the Cambrian period, possess antennae and antennal lobes reportedly containing glomeruli (8), and similar olfactory apparatus is nearly universal among the insects.

The remarkable similarity of glomerular organization in the first-order central olfactory neuropils of essentially all invertebrates and vertebrates that have a differentiated olfactory system has been noted often (e.g., refs. 9–12). Indeed, Dethier (1) argued persuasively that olfactory systems similar to those of contemporary insects and vertebrates, with comparable glomerular organization, were probably already in place 500 million years ago. Referring to environmental odor substances produced, first by photosynthesis and later by mankind's organic chemistry, in varieties far exceeding what could have been "anticipated" by evolving olfactory systems, Dethier observed (1):

> The ability of olfactory systems to cope with this plenitude of stimuli together with the fact that specific volatile compounds became associated with different plants and animals and different body sites, glands, and metabolites, provided exquisitely sensitive and accurate cues to the identities of places, trails, individuals, prey, predators, mates, social groups, and food. Olfaction permitted the development of a heretofore unparalleled perceptual talent.

The importance of that "talent" is evident in the facts that behavior mediated by olfaction is often concerned with intraspecific and interspecific communication or recognition and that olfactory input can have profound effects on the behavioral state of the animal (11).

Comparative and phylogenetic considerations such as those outlined in the preceding paragraphs readily lead to speculation that the olfactory systems of modern animals share common antecedents and therefore probably also share common principles of functional organization and information processing. We might ask, What attributes of chemical stimuli do olfactory systems analyze and encode? How are those features mapped in "neural space" at various levels of the olfactory pathway? How are cells of the pathway organized, and what mechanisms do they use, to accomplish this analysis of odors in the environment?

From Stimulant Molecule to Molecular Images in the Olfactory System

The breadth, precision, and behavioral significance of olfaction result from both peripheral and central mechanisms. The ability of olfactory systems to distinguish myriad odors depends on the response characteristics of ORCs, and hence ultimately on the cascades of molecular and cellular events, leading from molecular recognition at the receptor site to the generation of action potentials in temporal (in each ORC axon) and spatial (across the array of ORC axons) patterns that represent features of the stimulus. That ability also depends on neural circuitry in the CNS through which afferent olfactory information is integrated, abstracted, and recognized. To begin to understand how chemical signals are analyzed by the olfactory system and ultimately affect behavior, we must consider the anatomical and functional organization of the olfactory pathway and the processing performed, and abstraction accomplished, by neural circuitry at each level in that pathway.

Again this review emphasizes stimulating ideas put forth in R. H. Wright Lectures, in this case those of Gordon Shepherd (13). One of his key points is that an understanding of the neurobiology—the organization and function of neural circuits—of the olfactory system is crucial for relating properties of an olfactory stimulus to an animal's behavioral responses to it. Shepherd focused on vertebrate olfaction, but here we consider some aspects of his conceptual framework that appear to have much wider phyletic application. According to this model, olfactory information processing involves generation of a sequence of activity maps, termed "molecular images," in the olfactory pathway. Most of the mechanisms involved in this sequence are adaptations of, and bear similarities to, those underlying vision, immune responses, hormonal communication, chemotaxis of motile unicellular organisms, and other biological processes.

Common Features of Organization of Olfactory Systems. Olfactory systems of diverse vertebrates and invertebrates have certain general organizational and functional features in common. The pathway begins with ORCs residing in an epithelium and interspersed with supporting cells. Ciliary processes of ORC dendrites are relatively exposed, with only aqueous perireceptor fluid [e.g., mucus in vertebrates and sensillum liquor in insects (14)] separating the dendritic membrane from environmental chemicals. Odor molecules must traverse that aqueous phase, perhaps carried by odorant-binding proteins (14), to reach receptor sites in the ciliary dendrites of ORCs. Sensory transduction is believed to be initiated by binding of odor molecules to ORC membrane receptor proteins that are coupled to guanine nucleotide-binding proteins, triggering concatenated events involving multiple intracellular second messengers that ultimately open ion channels and thus generate a receptor potential in the ORC (15). The receptor potential spreads through the dendrite toward the cell body of the ORC and sets up a discharge of action potentials that propagate along the ORC axon to the CNS.

At their entry into the first-order olfactory center in the CNS (e.g., the olfactory bulb in vertebrates and the antennal lobe in insects), fascicles of ORC axons intermingle, and the axons defasciculate and refasciculate before terminating in glomeruli. This regrouping of primary-afferent fibers as they approach their central targets apparently accomplishes a reorganization of the axons, from grouping based on somatotopy to grouping based on odotopy. Each glomerulus encloses arborizations of central neurons that can be classified

into numerous types but generally fall into two main classes: projection (or principal or output) neurons that extend axons to subsequent way stations in the pathway and local interneurons confined to the olfactory bulb or lobe. Each ORC axon projects to one glomerulus, and many ORC axons converge on each glomerulus, where they have synaptic connections with neurites of particular types of central neurons (16, 17). On the basis of quantitative estimations performed in a number of vertebrate and invertebrate species (18), the ranges of orders of magnitude of elements associated with the first-order olfactory center appear to be 10^5–10^8 ORC axons projecting into an array of 10–1000 glomeruli, from each of which 1–100 projection neurons relay synaptically processed information about odor stimuli to higher centers in the brain.

Examples of projection neurons are the mitral and tufted cells of the vertebrate olfactory bulb, each with its principal dendrite confined to one glomerulus, and the uniglomerular and multiglomerular projection neurons of the insect antennal lobe. These neurons convey information about odor stimuli, as patterns of action potentials shaped through intra- and interglomerular synaptic circuitry involving various types of local interneurons as well as the projection neurons themselves, to higher order olfactory foci in the CNS (e.g., olfactory cortex in vertebrates, mushroom bodies and lateral protocerebrum in insects) (17, 19). Vertebrate olfactory cortex exhibits circuit properties that prompted Haberly (20) to suggest that olfactory cortex serves as a content-addressable memory for association of odor stimuli with memory traces of previous odors. In view of the striking parallel fiber arrays that characterize both pyriform cortex (20) and the mushroom bodies of insects (21, 22), it is not unreasonable to imagine a similar role for the latter structures. Moreover, mounting evidence suggests that interneuronal circuitry in other regions of the insect protocerebrum shapes descending premotor neural activity. This olfactorily influenced neural activity ultimately participates in the control of behavioral responses to odor stimuli (such as the characteristic flight patterns of male moths stimulated by female sex pheromone; ref. 23; see below).

The Primitives of Olfaction. To begin to understand how the olfactory system constructs molecular images or maps of information about odor substances in neural space, we must consider what properties—or primitives—of the stimulus molecules are being mapped (13). Among the molecular "determinants" that contribute to the "odogenicity" of an odor substance are molecular properties such as presence of functional groups, geometry (e.g., molecular length, position of functional groups, geometry of double bonds), and connectivity (e.g., number and sizes of rings and branching). The ability of odor stimuli to elicit behavior or evoke perceptions depends on multiple molecular properties of the stimulus molecules, including these individual molecular properties or determinants, population properties of a single odor substance, and appropriate mixtures of different odor substances (13). Also important, in addition to such qualitative properties, are odor intensity (concentration of odor substances) and intermittency. A requirement for interruption or intermittency of stimulation for effective olfactory function is found in diverse animals, from crustaceans and insects to air-breathing vertebrates (1).

Molecular Images in Olfactory Pathways. Pioneering efforts to understand the nature of olfactory coding were reported by Adrian (24–27). His work introduced the ideas that different odors activate ORCs in different regions of the olfactory epithelium and that spatiotemporal patterns of ORC firing would suffice to encode different odors. Subsequent studies by many investigators and involving various recording methods (reviewed in refs. 13 and 28) led to the conclusion that, at various levels of the pathway, the olfactory system uses distributed neural activity to encode information about olfactory stimuli.

Different odor substances stimulate different patterns of ORCs in the olfactory epithelium, owing to the different sensitivity spectra of the ORCs (28). The pattern of activity in the epithelium evoked by a particular odor substance constitutes the first molecular image of that stimulus, which represents the determinants of the stimulating molecules (13). Thus, although olfaction is not a spatial sensory modality, in contrast, for example, to vision and somatosensation, the initial representation of an odor stimulus in the olfactory pathway does have spatial structure.

At subsequent levels in the olfactory pathway, new molecular images of the odor stimulus are formed as patterns of activity across an array of neural elements. For example, in the olfactory nerve, which carries ORC axons to the olfactory bulb or lobe of the brain, the pattern of activity across the array of fasciculated primary-afferent fibers constitutes the second molecular image of the stimulus. In the olfactory bulb or lobe, another molecular image takes shape as the pattern of activity across the array of glomeruli, and yet another molecular image is generated as the pattern of activity across the array of projection-neuron axons emanating from the glomeruli. Each molecular image of a particular odor stimulus exemplifies the way neural space is used at that level in the pathway to represent information about the stimulus.

An important insight from many studies (28) is that the response patterns—the molecular images—at various levels in the central olfactory pathway are set up by the differential responses of the ORCs in the peripheral receptor epithelium. These studies also suggest that functional modules, which may correspond to recognizable structural units such as individual glomeruli with their associated cells, in the olfactory bulb or lobe participate in the analysis of olfactory information conveyed to them by primary-afferent ORC axons (28). A characteristic set or pattern of modules would be activated by a given odor stimulus, and particular modules could be shared by the patterns activated by different odor stimuli if the molecular determinants of the stimuli overlap. Thus, for example, experiments using the radioisotopic 2-deoxyglucose method of activity labeling indicated that different odors evoke activity in glomeruli localized in different regions of the olfactory bulb (29). Moreover, recent investigations of the response specificities or "molecular receptive ranges" of individual uniglomerular output neurons (mitral and tufted cells) in the vertebrate olfactory bulb strongly support the idea that the glomeruli are functional units (30–33).

A Case in Point: Neural Processing of Sex-Pheromonal Information in Moths

In many species of insects, olfaction is decisive for the control of several kinds of behavior. Orientation and movement toward, and interactions with, potential mates, appropriate sites for oviposition, sources of food, and hosts for parasitism often involve olfactory signals that initiate, sustain, and guide the behaviors. Because of their prominence in the zoosphere, their economic and medical importance, and their usefulness as models for both behavioral and neurobiological research, insects have been studied extensively to elucidate mechanisms of olfactory control of behavior. Insects respond to a variety of semiochemicals, including pheromones and kairomones. Studies of the responses of insects to such biologically significant odors have shown that the quality and quantity of odor substances in complex mixtures present in the environment are encoded in patterns of activity in multiple ORCs in the antennae. These "messages" are decoded and integrated in the olfactory centers of the CNS and ultimately lead to olfactorily induced changes in the behavior of the insect.

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 signal with well-characterized mate-seeking behaviors involving arousal, patterned upwind flight, short-range orientation to the calling female, and mating (34, 35).

Building upon the work of others (36–39) and paralleling current research in other laboratories on different insect species, we study the olfactory system of the experimentally favorable giant sphinx moth *Manduca sexta*. The brief review presented here focuses on the functional organization and physiology of a sexually dimorphic olfactory subsystem in this species [also reviewed elsewhere (19, 23, 40–42)].

The principal long-term goals of this line of research are to understand the neurobiological mechanisms through which the conspecific females' sex pheromone is detected and information about it is integrated with inputs of other modalities in the male moth's brain and to unravel how the message ultimately initiates and controls his characteristic behavioral responses. Pursuit of these goals promises to teach us much about how the brain processes olfactory information and uses it to shape behavior. Our studies to date have persuaded us that the male's olfactory system consists of two parallel subsystems: one is a complex, sexually isomorphic pathway that processes information about plant (and probably other environmental) odors encoded in "across-fiber" patterns of physiological activity and bears striking similarities to the main olfactory pathway in vertebrates, and the other is a sexually dimorphic "labeled-line" pathway specialized to detect and process information about the sex pheromone.

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 biosynthesis and modification of fatty acids (34). Often, a species-specific blend of components is the message, and males of many moth species, including *M. sexta*, give their characteristic, qualitatively and quantitatively optimal behavioral responses only when stimulated by the correct blend of sex-pheromone components and not by individual components or partial blends lacking key components (43, 44).

Solvent washes of the pheromone gland of female *M. sexta* yield eight C_{16} aldehydes (as well as four C_{18} aldehydes believed not to be pheromone components) (44). A synthetic mixture of the C_{16} aldehydes elicits the same behavioral responses in males as does the signal released by a calling female (44, 45). A blend of two of the components, the dienal (*E*,*Z*)-10,12-hexadecadienal and the trienal (*E*,*E*,*Z*)-10,12,14-hexadecatrienal—hereinafter called components A and B, respectively—elicits an apparently normal sequence of male behavior in a wind tunnel, but the individual components are ineffective (44). Field trapping studies have shown that a blend of the eight C_{16} aldehydes is significantly more effective in attracting males than are blends of fewer components (46), suggesting that all eight C_{16} aldehydes play roles in the communication system of *M. sexta*—i.e., that the sex pheromone of this species is composed of those eight C_{16} aldehydes (44, 45).

Our neurophysiological studies have focused on three 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 (47, 48)]. 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 A and B are present in the blend (44), when the concentrations and blend proportions of the components fall within acceptable ranges (49), and when the pheromone blend stimulates his antennae intermittently (39, 50). In our studies, 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. The distalmost, third segment of the antenna of adult *M. sexta* is a long, sexually dimorphic flagellum divided into at least 80 annuli bearing numerous sensilla of several types, the great majority of which are olfactory (51, 52). Antennal flagella of both male and female *M. sexta* have many ORCs that respond to volatile substances given off by plants (53) and presumably are involved in host–plant recognition and discrimination. In addition, the flagella of the male moth possess ORCs specialized to detect the individual key components of the female's sex pheromone (53, 54).

A male flagellum has $\approx 3 \times 10^5$ ORCs associated with $\approx 10^5$ recognized sensilla, of which $\approx$40% are male-specific sensory hairs or sensilla trichodea (51, 53–56). Type I trichoid hairs ($\leq$600 μm in length) are typical olfactory sensilla, with single walls and pores, and include two ORCs that send their unbranched dendrites through the lumena of the fluid-filled hairs to their tips (51, 55–57). In most of these sensilla, one of the two male-specific ORCs is highly sensitive and specific to pheromone component A, while the second ORC is tuned to component B (54). Pheromone-specific ORCs in moth antennae thus represent information about stimulus quality by means of their specialization as narrowly tuned input channels. Groups of these cells can "follow" intermittent pheromonal stimuli at naturally occurring frequencies ($\leq$10 stimuli per sec) (58).

Olfactory Transduction in Pheromone-Specific Receptor Cells. Physiological and biochemical approaches have yielded increasingly detailed information about mechanisms underlying the transduction of odor stimuli into electrophysiological activity in antennal ORCs (59). In the case of *M. sexta*, primary cell cultures of immunocytochemically identifiable, male-specific ORCs have been studied by means of patch-recording and pharmacological techniques (60–63). These cultured ORCs respond to stimulation by pheromone components with opening of nonspecific cation channels that appear not to be directly gated by pheromone receptors but, instead, to depend on intracellular second messengers mobilized via receptor-coupled guanine nucleotide-binding protein(s). The possibility that these cation channels are controlled by one or more intracellular messengers is consistent with findings from biochemical studies on other species (59). It is likely that these cation channels, which are permeable to Na^+ and K^+ as well as Ca^{2+} ions, play an important role in pheromone transduction.

Functional Organization of Central Olfactory Pathways. Axons of antennal ORCs project through the antennal nerve to enter the brain at the level of the ipsilateral antennal lobe (AL) of the deutocerebrum (52). ORC axons project from the flagellum to targets in the AL, but axons from antennal mechanosensory neurons bypass the AL and project instead to an "antennal mechanosensory and motor center" in the deutocerebrum posteroventral (with respect to the body axis of the animal) to the AL (52, 58, 64). In moths and certain other insect groups, sex-pheromonal information is processed in a prominent male-specific neuropil structure in each AL called the macroglomerular complex (MGC) (16, 52, 64, 65).

In *M. sexta* the AL has a central zone of coarse neuropil (largely neurites of AL neurons) surrounded by an orderly array of glomeruli, including 64 $\pm$ 1 spheroidal "ordinary" glomeruli and, in the male, the sexually dimorphic MGC near the entrance of the antennal nerve into the AL (64, 66). Bordering this neuropil are the lateral, medial, and anterior groups of AL neurons, totaling about 1200 cells (19, 65, 67). The ordinary glomeruli, which are condensed neuropil struc-

tures 50–100 μm in diameter, contain terminals of sensory axons and dendritic arborizations of AL neurons, as well as primary-afferent synapses and synaptic connections among AL neurons, and they are nearly surrounded by glial cells (65, 68, 69). Each ORC axon from the antenna terminates within a single glomerulus in the ipsilateral AL (64, 68, 89), where it makes chemical synapses with neurites of AL neurons, primarily local interneurons (65, 68, 70, 71).

With very few exceptions, the neurons in the medial, lateral, and anterior cell groups of the AL fall into two main classes (19, 65, 67, 72, 73). 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 olfactory foci in the protocerebrum (67, 72). Many PNs have dendritic arborizations confined to single AL glomeruli and axons that project via the inner antennocerebral 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 (67, 73, 74). Other PNs have arborizations in one or more AL glomeruli and send axons via different antennocerebral tracts to characteristic regions of the lateral and inferior protocerebrum (19, 67, 73).

Axons of male-specific antennal ORCs specialized to detect components of the sex pheromone project exclusively to the MGC (64, 89), and all AL neurons that respond to antennal stimulation with sex pheromone components have arborizations in the MGC (65, 72, 73). The MGC in *M. sexta* has two major, easily distinguishable divisions: a donut-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 (74). AL PNs that respond to antennal stimulation with sex pheromone component A have arborizations in the toroid and PNs responsive to component B, in the cumulus (74). Thus first-order synaptic processing of sensory information about these key components of the sex pheromone apparently is confined to different, distinctive neuropil regions of the MGC.

Stimulus Quality. By means of intracellular recording and staining methods, we have examined the responses of AL neurons to stimulation of the ipsilateral antenna with each of the sex pheromone components as well as partial and complete blends (75). In accordance with results of behavioral and sensory-receptor studies, components A and B are the most effective and potent sex pheromone components for eliciting physiological responses in the male-specific AL neurons. On the basis of these responses, we classified the neurons into two broad categories: pheromone generalists and pheromone specialists (76). Pheromone generalists are neurons that respond similarly to stimulation of either the component A input channel or the component B input channel and do not respond differently when the complete, natural pheromone blend is presented to the antenna. In contrast, pheromone specialists are neurons that can discriminate between antennal stimulation with component A and stimulation with component B. There are several types of pheromone specialists. Some receive input only from the component A input channel or the component B input channel and thus preserve information about individual components of the blend.

An important subset of pheromone-specialist PNs in male *M. sexta* receives input from both component A and component B input channels, described above, but the physiological effects of the two inputs are opposite (72). That is, if antennal stimulation with component A leads to excitation, then stimulation with component B inhibits the interneuron, and vice versa. Simultaneous stimulation of the antenna with both components A and B 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 PNs also respond uniquely to the natural pheromone blend released by the female: these pheromone specialist neurons have enhanced ability to follow intermittent pheromonal stimuli occurring at natural frequencies of ≤10 stimuli per sec (77).

Stimulus Quantity. Numerous studies in the field and in wind tunnels have shown that pheromone-mediated orientation is dose-dependent (35). We therefore have examined the ability of AL neurons to encode changes in the concentration of a pheromonal stimulus (76). 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 equivalent (FE) of sex pheromone component(s) in the odor-delivery source], 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, measured in terms of the number of spikes elicited, that is quite linear up to 0.05 FE but falls off above this concentration. The maximum instantaneous frequency of spiking, however, continues to increase up to the highest concentration tested (0.1 FE). A corresponding increase in the amplitude of the membrane depolarization can also be seen.

Stimulus Intermittency. A third important characteristic of a female moth's sex-pheromone plume is its nonuniformity. Simulation of odor plumes using ionized air has shown clearly that a plume is not a simple concentration gradient but instead is distinctly filamentous and discontinuous (47, 48). 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 (35). Because spatial discontinuity of the pheromonal signal in the environment is detected by a flying male moth as temporally intermittent stimuli, intermittent pheromonal stimuli received by a male's antenna must be registered by MGC PNs. We discovered that certain pheromone specialist PNs have greatly enhanced ability to follow pulsed pheromonal stimuli with corresponding bursts of impulses. These are the PNs cited above that can discriminate between components A and B because one of these key components excites the cells while the other inhibits them. This inhibitory input to such PNs enhances their ability to follow brief pulses of pheromone blends delivered at frequencies up to 10 stimuli per sec by controlling the duration of excitatory responses and preparing the PN for the next bout of excitation (77).

Synaptic Mechanisms in the AL. Having characterized many AL neurons both morphologically and physiologically, we have sought to explain how their characteristic patterns of responses to olfactory stimuli are generated. To accomplish this mechanistic goal, we must analyze the synaptic "wiring" of the AL, test physiologically for synaptic interactions between known types of AL neurons, identify neurotransmitters and synaptic mechanisms employed by AL neurons for intercellular communication, and seek evidence for and mechanisms of integration of other modalities with olfactory inputs in the ALs.

Synapses between ORC axons and their AL target neurons are excitatory and appear to be mediated by the neurotransmitter acetylcholine acting through nicotinic cholinoceptive mechanisms (40, 71, 78–82). Another prominent neurotransmitter in the ALs is γ-aminobutyric acid (GABA) (79, 83). GABA immunocytochemistry has revealed that all of the GABA-immunoreactive neurons in the AL have somata in the large lateral cell group of the AL (84). There are ≈350 GABA-immunoreactive LNs and 110 GABA-immunoreactive

PNs (i.e., ≈30% of the neurons in the lateral cell group may be GABAergic) (19, 84). Most (and possibly all) of the LNs are GABA-immunoreactive and thus may be inhibitory interneurons. The important inhibitory postsynaptic potential (IPSP) that enables certain pheromone specialist MGC PNs to follow intermittent pheromonal stimuli (see above) appears to be due to chemical-synaptic transmission mediated by GABA (85). This IPSP reverses below the PNs' resting potential and is mediated by an increased Cl^- conductance. This IPSP can be inhibited reversibly by picrotoxin, which blocks GABA receptor-gated Cl^- channels, and by bicuculline, a blocker of vertebrate $GABA_A$ receptors. Furthermore, applied GABA hyperpolarizes the postsynaptic neuron, and this response can be blocked reversibly by bicuculline, indicating that bicuculline directly blocks the GABA receptors. Such GABAergic synaptic transmission is essential to the enhanced ability of the specialized AL PNs to follow intermittent pheromonal stimuli (77).

Synaptic Interactions Between AL Neurons. We have tested the idea that this inhibition of PNs is mediated through LNs by directly recording synaptic interactions between pairs of AL neurons (70). Current was passed into one neuron while the postsynaptic activity in the other neuron was monitored. None of the PN–PN pairs examined showed any current-induced interactions, but a significant proportion of the LN–PN pairs studied exhibited such interactions, all of which were unidirectional. That is, LN activity could influence PN activity, but not vice versa. Depolarizing current injected into an LN, causing it to produce spikes, was associated with cessation of firing in the PN. Spike-triggered averaging revealed a weak, prolonged IPSP in the PN. Cross-correlation analysis also revealed a weak inhibitory interaction, polarized in the direction from LN to PN. When the simultaneous responses of the two neurons to olfactory stimulation of the ipsilateral antenna with pheromone component A were recorded, a brief period of inhibition was observed in the LN, and this was followed shortly thereafter by a transient increase in the firing frequency of the PN. This suggests that the "excitation" of the PN is due to disinhibition. The LN that synaptically inhibits the PN thus may itself be inhibited by olfactory inputs, probably through another LN.

Higher Order Processing of Pheromonal Information in the CNS. 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 PNs with arborizations in the MGC. 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 (86, 87).

Many pheromone-responsive protocerebral neurons have arborizations in the lateral accessory lobes (LALs), which are situated lateral to the central body on each side of the protocerebrum and appear to be important for processing of olfactory information (86). Each LAL is linked, by neurons with arborizations in it, to the ipsilateral superior protocerebrum as well as the lateral protocerebrum, where axons of AL PNs terminate (67, 72, 73). 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 complete sex pheromone blend or a mixture of components A and B than by either component alone. LLE in response to pheromonal stimuli was observed in >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. In *M. sexta*, AL PNs responding to pheromone components do not show LLE (72, 73). 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 neurons from olfactory foci elsewhere in 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 may be transmitted to thoracic motor centers. How this LLE might contribute to the generation of the male moth's characteristic behavioral responses to sex pheromone is currently unknown.

Conclusion. The male moth's pheromone-analyzing olfactory subsystem is composed of pheromone-specific antennal ORCs projecting to the similarly specialized, anatomically defined MGC in the AL and MGC output neurons that project to olfactory foci in the protocerebrum. This subsystem is an example of a labeled-line pathway (18). Its specialization to detect, amplify, and analyze features of sex-pheromonal signals and its consequent exaggeration of common olfactory organizational principles and functional mechanisms make this system especially favorable for experimentation as well as for computational modeling (88).

In this specialized subsystem, the molecular images attributable to at least the first several levels of the pathway seem, to a first approximation, to be relatively simple. For components A and B of the pheromone, the first molecular image at the level of the antennal olfactory epithelium would be a pattern of activity corresponding to the orderly anatomical pattern of distribution of type I trichoid sensilla on each annulus of the flagellum. At the next level, the molecular image would be a pattern of activity on a cross-section of the antennal nerve corresponding to the pattern of fasciculation of axons of ORCs specialized to respond to components A and B. At the level of the glomeruli of the AL, the molecular image would map isomorphically with the major subdivisions of the MGC, the component A-specific toroid and the component B-specific cumulus, and so forth through the MGC output tracts and protocerebral olfactory foci to descending premotor pathways, one can envision hypothetical molecular images of the pheromone at each successive level.

This way of viewing the olfactory system spotlights the kinds of information about odor stimuli to which the brain attends. For example, although the blend of components is essential to evoke and sustain the normal male responses to the sex pheromone, information about specific components is preserved through many levels of the pathway. Thus it appears that information about single components as well as their blend may be important for chemical communication in these insects.

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