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Chemical communication in invertebrates

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Chemical communication is pervasive in nature

In these modern times one feels relatively secure in stating that all biological events are ultimately chemical events in one form or another. Although it remains risky to attempt a similarly facile description of the symmetry breaking physical events which originally gave rise to the dynamic patterns and nonequilibrium states which define life's processes, it is clear that: living, better or otherwise, is chemistry. Thus the synthesis, transport, detection, utilization and storage of chemical compounds are essential capabilities of living organisms. In a similar vein, it is apparent that all of the organizing transformations which distinguish living matter, at whatever level of biological complexity one may wish to consider, require systems for the generation, transmission, reception, processing and storage of information. This capability to utilize information and to sustain its incessant flux between and within the various compartments of a living system determines and supports nature's emergent properties[17,29].

The animated communication systems found in nature have evolved to satisfy an enormous range of need and purpose. They vary widely in the media and mechanisms used for signaling and in the relative immutability of their messages both in time and in space. For example, a detailed set of instructions for the assembly of a complete individual is contained in the genetic material found in each of the component cells of an organism. This genetic information is coded directly in the complex chemical structure of the DNA molecule, where it is faithfully transmitted again and again over the course of evolutionary time[25]. Communicative features are also readily apparent in the detection and recognition of antigens by individual lymphocytes and in the subsequent conversion of the lymphocyte into a blast cell which can produce a whole new colony of cells each with a specific set of detectors for recognizing the original antigenic signal[33,41]. The evolution of creatures with discrete nervous systems, wherein chemical and electrical signals mediate, coordinate and modulate life's myriad component stages is an obvious example of the development of specialized biological communication systems which gather together into a unified structure many of the available modes of information transfer required for efficient communication both within and between individuals. This developmental process culminates in the appearance of human intellect and language with their profound effects on the organization and regulation of human behavior.

Chemical compounds can be tailored in a seemingly endless variety of ways to produce chemical signals with a wide variety of spatial and temporal properties. It is not too surprising therefore to find that the common problems on inter- and intra-specific communication have been met most often with the tools already at hand, and have resulted in the frequent evolution of chemical communication systems. These systems range from the generalized ability of the *E. coli* bacillus to respond chemotactically to a wide variety of different environmental chemicals[1,20], to the more specialized communication between pre- and post-synaptic membranes in the vertebrate neuromuscular junction which utilizes a relatively small number of specific chemical messengers[28].

Chemical communication system design

It is a generally accepted principle that the evolutionary significance of a particular message is positively corre-

lated with the specificity and fidelity of the communication system within which it is processed. Therefore, chemical communication systems, in which these factors may attain full expression are often found to be intimately involved with those organismic processes which involve reproduction, either directly or indirectly. Reproductive capabilities have primal importance for the survival of any species. Thus, a chemical recognition system is found to regulate the interaction of sperm and egg so that ova are routinely fertilized by sperm from the appropriate species, even when sperm from a variety of organisms are artificially allowed to compete directly for the same ova[39]. Specific olfactory signals are used in insects for a variety of important purposes such as trail markers, sex attractants and aphrodisiacs; for alerting, alarming and aggregating other individuals and in the selection of mates, food sources and oviposition sites[5,22,23]. In a like manner, unique chemical signals in a vertebrate like the hamster are known to modulate such important behaviors as mating, territorial aggression and scent marking[16,35]. The mythology surrounding the role of certain aromas in enhancing human sexual behavior is extensive and has fostered a multi-billion dollar market for perfumes and deodorants.

The control of physiological processes and behavioral states by way of chemical messages manufactured and secreted by one organism to be received by another, was first clearly documented in invertebrates, especially insects. These chemical messages are called pheromones and are of at least two types: the primer pheromones which affect the endocrine system and often result in profound changes in the receiving animal's reproductive capabilities and the stimulus response pheromones which cause more or less immediate changes in the animal's behavior[5,8,18]. We will consider here only the latter type of chemical signal although many of the statements made regarding them may also be applicable to primer pheromones.

In order to provide a theoretical framework within which to evaluate the sensory mechanisms involved with the perception of olfactory signals, consider for a moment the incredible range and diversity of chemical compounds present in the environment of an evolving organism which ultimately develops a chemical communication system. At the molecular level exceedingly small changes in structure such as differences between optical isomers are to be discriminated. The molecular diversity that must be handled by the system continues to increase as other organisms, including organic chemists, synthesize new compounds that could never have existed before[40]. The concentration of these potential chemical signals may vary from as little as 1 molecule/cc of medium up to hundreds of trillions of molecules/cc. To cope with this an animal needs a detection system with enormous gain so that very dilute stimuli like those emanating from potentially willing but remote sexual partners will be detected. Moreover, its resolving power must be prodigious because important signals will probably be buried in a sea of other, non-significant, compounds. The system should also have a very wide dynamic range so that small differences in local concentration gradients may be used to locate the sources of distant chemical signals. Finally all of these requirements must be packed within the space allocated to the nervous system and fueled within the energy budget imposed by the overall metabolic cost of existence.

These diverse requirements are not readily satisfied by a single solution but may be accomplished by designing two or more different, but interrelated olfactory communication systems. In one, a set of broad range detectors and their central connections are responsible for discriminating amongst a very large number of potentially important odor signals and in the second, a narrow band, high sensitivity system of detectors and their central neural connections are responsible for detecting dilute chemical signals which have very large amounts of survival value such as those having to do with reproduction. These systems may operate independently or they may be mixed or chained together so that discriminations made in one, may affect the discriminations made in the other.

Although there are many gaps in our knowledge, a number of related observations exist concerning the general organization of chemical communication systems in both vertebrates and invertebrates which suggest that the partitioning of function into discrete components may have a basis in fact. Both vertebrates and invertebrates have morphologically distinct or anatomically segregated receptor neuron populations. For example insects have both trichodeal and basiconical olfactory sensilla, while many vertebrates have both main olfactory and vomeronasal receptor organs. Both vertebrates and invertebrates respond to at least two general classes of olfactory signals, those which originate with conspecifics such as sex pheromones, and those which are of more general interest such as food odors. In the case of insects and probably also in vertebrates the two distinct classes of receptor neurons seem to be preferentially devoted to the two classes of stimuli. Electrical activity in these two sensory systems seems to lead on the one hand to reproductively linked behaviors and on the other to generalized chemical sensitivity and behaviors related to this capability such as orientation and food seeking.

Chemical communication in insects

Here we shall be interested in only those chemical communication systems in insects where odor signals exert their effects through the olfactory system and give rise to sex related behaviors. Invertebrate neurobiologists once thought that such pheromones were simple, highly specific chemical signals which would evoke a specific behavioral response on the part of the receiving individual and that they would be effective in controlling rather general aspects of an insect's behavior[51]. However, it is now apparent that pheromone communication systems are highly complex and sophisticated communication networks influencing many aspects of an individual insect's life, its relationship with other members of its own and in some cases, members of different species[7,23]. It has also become apparent that the pheromones themselves are not simple odor sources[15]. For instance, appropriate behavioral responses are known to be elicited by a single chemical compound, by different intensities of a single compound, by a specific medley of several individual compounds, by varying the proportions of com-

pounds in such a medley and finally by various combinations and permutations of all of the above. In addition to this complexity it is now clear that certain components of the natural pheromone used by one insect species can modulate the normal behavioral response of another species to its own pheromone complex[13, 44, 45]. Even a seemingly simple behavioral response like capturing a male moth in a pheromone baited trap is differentially influenced by a range of different pheromone components and analogs and thus may be subdivided into several different intervening behavioral steps[10, 24]. In short all the behavioral and chemical evidence gathered to date indicates that both pheromones themselves and the pheromone communication system of different insects are highly organized, complex and often interactive.

Given the complexity of the pheromone systems involved, and the well-founded desire to use them as an ecologically sound method of insect pest control, it is absolutely necessary that the neural mechanisms underlying, and responsible for, pheromone perception become the objects of intensive study. This need is particularly acute in the case of the sex pheromones because preliminary experiments have shown that these compounds can be used to effectively control the size of an insect population[9, 42, 46].

One of the basic problems to be evaluated may be stated quite simply: given that the olfactory communication systems of insects are, in nearly all cases, relatively sophisticated, with multiple chemical signals conveying a variety of interrelated messages which ultimately result in a series of integrated, context specific behavioral responses; how does the insect nervous system detect, encode and process these messages unambiguously and still cope with the range of theoretical restraints enumerated earlier? An initial step toward answering this admittedly global question involves a consideration of the specific response properties of individual insect olfactory receptors when presented with a range of different olfactory stimuli. It is axiomatic that all of the relevant chemical signals needed for the behavioral process at hand are detected and encoded by chemosensory receptor neurons and that the design of the neural apparatus is appropriate for the perception at hand. Thus peripheral encoding of sensory information must occur even in those instances where further central integration with information, obtained from other sources alters or blocks expression of the usual behavioral response. After all, little survival value accrues to an organism which attempts to reliably produce different behavioral responses with receptor neurons which cannot discriminate between the relevant chemical signals.

At this point it should be emphasized that any attempt to consider the actual perception of insect pheromones must include several disclaimers. First, no information exists concerning the mental processes (if such exist) evoked in an organism by pheromonal stimulation. This of course prevents any consideration of the actual mental state or perceptual mechanism which comes into play when these physical stimuli are evaluated by the senses. In addition, those descriptions of the neural mechanisms which operate in the sensory portions of an insect nervous system which are available to us, are hindered in most cases because they have been obtained in a range of different species using a variety of different chemical signals and a wide range of response measures. These descriptions are further hampered by an inexact knowledge of the portions of the animal's neuropil which are actually involved with processing the particular sensory information under consideration. Because of these factors there is as yet no single organism in which sufficient information exists at each of the relevant neural levels for an adequate description of the perception of pheromones. Therefore we will further restrict the scope of our discussion and present a small sampling of results, largely from work in Lepidoptera, which indicates the basic framework within which this area of inquiry can be considered and serves to highlight the general features of chemical communication in invertebrates.

A) Chemical signals and odor-guided behavior

Without belaboring the historical background it is clear that our appreciation of the chemical signals used by insects and the range of behavioral responses occasioned by such stimuli has expanded greatly in the last 25 years. The original working hypothesis that each insect produced a one component pheromone which in turn elicited a single behavioral response in a receiving animal[48] has given way to one characterized by considerable complexity with regard to the number of chemical signals present in the secretion of a single pheromone gland[50], the range of behaviors modulated by these stimuli[24, 50] and whether these compounds act individually[7] or in groups[24]. With the advent of modern microchemical techniques, with their increased sensitivity and resolution, it is now possible to directly measure the volatile chemical signals produced by single calling females[3, 47].

The majority of species examined in this way produce multicomponent pheromone blends and where the behaviors elicited by these blends have been evaluated in natural or seminatural conditions, a range of different odor-guided behaviors has been observed[2, 5, 7, 24, 50]. Although the total range of communicative complexity is only dimly perceived, because of the small number of species yet evaluated, present knowledge does illuminate some of the relevant factors which are likely to be general features of pheromone communication systems.

Females of both the redbanded leafroller moth, *Argyrotaenia velutinana* (RBLR), and the cabbage looper moth, *Trichoplusia ni* (CL), produce multicomponent pheromones. Although the exact number of behaviorally important compounds may yet be revised upward, gland extracts in both species routinely contain a relatively large number of different 12–18 carbon saturated and unsaturated fatty acids, acetates and perhaps alcohols. Some of these chemicals are behaviorally relevant components of the pheromone, others are likely percursors for these compounds and the remainder have yet to be explained[6].

The RBLR female produces at least three behaviorally relevant pheromone components: Z-11-tetradecenyl acetate (Z11–14 : AC), E-11-tetradecenyl acetate (E11–14 : AC) and dodecyl acetate (12 : AC)[47]. Collectively these compounds are powerfully attractive to males. Extensive field and laboratory observations of male behav-

ior by Roelofs and his colleagues have begun to dissect the various steps in a male's response to a calling female and the relative influence of the various pheromone components in these individual behaviors[2,4,44,45,47]. Exposure of males to any of the three pheromone components, at intensities appropriate to the natural situation, fails to elicit significant male response[2]. Very large amounts of any one of the components (10–1000 µg) can elicit wing fanning, which is one of the earlier observable male responses to pheromone. However, subsequent stages in the behavioral sequence which culminates with copulation are usually not obtained with single purified components. When the two tetradecenyl acetate isomers are mixed in a ratio which approximates that found in the female gland (92% Z11–14 : AC, 8% E11–14 : AC), males at rest reliably engage in the normal early activation behaviors (antennal elevation and wing fanning) and then take off and fly upwind. Although there is some suggestion from the data that Z11–14 : AC is more important for flight initiation, whereas E11–14 : AC is more important for the maintenance of upwind flight, it is clear from these studies that the relative ratio of the two compounds is the most important determinant of behavioral activation. Significant reductions in behavioral response occur with relatively small changes in the ratio of isomers[47]. Thus it appears that these two isomers in concert act as a single chemical message signaling flight initiation and maintenance. The third component of the RBLR pheromone, 12 : AC, modulates the landing frequency of males aloft, in proportion to its intensity relative to the tetradecenyl acetates (optimal ratio 3 : 2). It should be noted that several environmental non-olfactory signals are known to influence the behavioral outcome of pheromonal stimulation. These include apparent ground motion, wind speed and direction and the relative size of the pheromone source[2,21]. In the latter instance the incidence of landing and close range orientation to a source is enhanced both by the addition of 12 : AC to an artificial pheromone blend and by reducing the size of the landing surface available to the male.

The pheromonal communication system seems even more complicated in the CL where at least 6 behaviorally relevant odor components have been demonstrated in the female pheromone gland[6,24]. These compounds and their relative proportions include: Z-7-dodecenyl acetate (Z7–12 : AC, 100%), Z-5-dodecenyl acetate (Z5–12 : AC, 7.6%), 12 : AC (6.8%), 11-dodecenyl acetate (11-12 : AC, 2.3%); Z-7-tetradecenyl acetate (Z7–14 : AC, 0.9%) and Z-9-tetradecenyl acetate (Z9–14 : AC, 0.6%). Although it is clear that Z7–12 : AC is essential for flight initiation, the participation of the 5 remaining components in eliciting one or another of the remaining range of male behaviors normally observed in response to calling females is not easily parsed into the usual chemical signal, behavioral response sequences. All six compounds act together to elicit male behaviors indistinguishable from those obtained in response to a female. Except for Z7–12 : AC, one or two of the compounds can be removed, more or less at random, from the pheromone blend without noticeably reducing communicative efficiency. Further reductions in the complexity of the blend begins to selectively decrease the occurrence of the later portions of the behavioral sequence. These include: male hair-pencilling, contact with the pheromonal source and maintenance of upwind flight. However the exact combination of compounds removed does not seem to preferentially influence one or another of these behaviors. In fact, 6 of the 10 theoretically possible 4 component mixtures elicited peak responses from test males which were indistinguishable from those obtained by calling females. Even the 4 remaining, less effective blends, still elicited relatively normal numbers of flights and upwind orientations. Thus, there is a great deal of redundancy in the exact composition of the chemical signals effective in this animal and this redundancy seems to be expressed at all of the steps in the behavioral response except for the unique requirement for Z7–12 : AC in flight initiation[6,24]. It should be clear from these two examples that in spite of the general uniformity of the chemical types employed, it is the exact composition of the pheromone bouquet which determines the unique species specific capabilities of this form of chemical communication[43]. Even in those cases (e.g. 12 : AC) where the same compound is important in modulating male responsiveness in different species, the exact behavior effected and its staging in the particular behavioral sequence which culminates in copulation may still be unique. These properties of the system and their known interactions with other environmental and physiological variables lead us to next consider the anatomical facilities which are generally available in this sensory domain and how their functionality may begin to account for chemical communication in invertebrates.

B) Chemical receptors and methods of study

Although the exact morphological details vary from species to species[27], figure 1 (A–C) illustrates most of the common surface features expected on the male antennae. Each antenna is a complex, segmented sensory organ equipped with mechanoreceptors and various types of chemoreceptors. The chemoreceptors associated with pheromone perception are usually found on the antennae in morphologically distinct[36] sensilla (fig. 1B) but additional chemoreceptors may be distributed over other body regions. Each sensillum contains two or more olfactory receptor neurons which communicate with the outside world through minute pores in the cuticle (fig. 1C)[14,49]. These pores presumably allow odor molecules to interact with the dendritic portions of the receptor neuron membrane contained within the sensillum. Although the receptor neurons are sister cells developmentally, they often have widely different response properties.

In most experimental studies, olfactory pheromones are usually directed onto the antennae from odor cartridges which are loaded with microgram amounts of compound. Because of the relatively low volatility of most insect pheromones, the effective stimulus concentration actually dispensed from cartridges is unknown, but is assumed to be in the picomolar range. In some cases it has been estimated that only a few pheromone molecules may be sufficient for the production of a response in insect olfactory receptor neurons. The general experimental paradigm used in most studies of olfactory receptor neuron function includes immobilization of the exper-

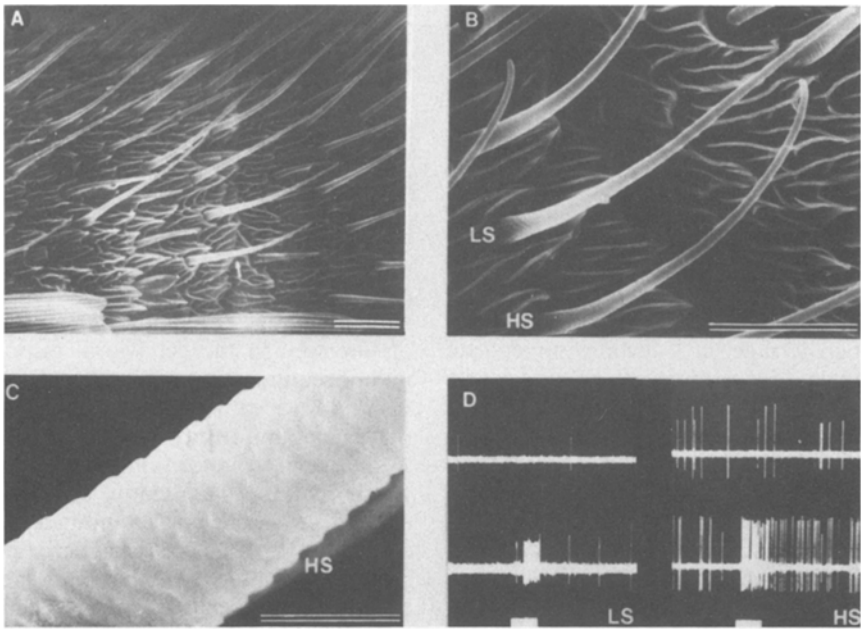

Figure 1. External morphology of male cabbage looper moth antennae (A–C) and electrophysiological recordings (D) from two classes of identified olfactory sensilla. *A* A low power (× 1100) scanning electron micrograph (SEM) showing the diversity of sensilla types on a subsegment of the antenna. Most of the hair-like sensilla in this view contain receptor neurons which respond to olfactory stimuli. *B* A medium power (× 3000) SEM of the distal margin of one subsegment on a male antennae. The sensillum labeled LS contains two receptor neurons with relatively low levels of spontaneous activity whereas the one labeled HS contains two receptor neurons with higher levels of spontaneous activity. *C* A high power (× 22000) SEM of a portion of the surface of the HS sensillum, indicated by the arrow in figure 1A, illustrating the annulations and cuticular pore density characteristic of this type. Scale marks are 10 μm in *A* and *B* and 1 μm in *C*. *D* Extracellular recordings from morphologically identified LS and HS sensilla showing in the top traces the characteristic differences in the spontaneous activity of their receptor neurons and in the bottom traces differences in these neurons' sensitivity to Z-7-dodecenyl acetate (Z7–12:AC), a component of the female sex attractant pheromone. In each sensillum the activity of the two receptor neurons may be distinguished by characteristic differences in action potential amplitude. The scale bar in the lower LS record indicates the application of a 2-s puff of pheromone from a cartridge containing 10 μg of Z7–12:AC. The scale bar in the lower HS record indicates a 2-second puff from a cartridge containing 0.01 μg of Z7–12:AC.

imental animal and fixation of the antenna[31, 32]. An indifferent electrode is inserted into the antennal blood space at the distal end of the antennae and a sharpened recording electrode is inserted at the base of the selected sensillum. Alternatively the tip of the sensillum is cut off and a saline filled electrode is positioned over the cut end to make electrical contact with the dendritic membranes. All electrode placements record biphasic action potentials from the several receptor neurons contained within the sensillum as is shown in figure 1D. The diameter of the dendritic processes within the lumen varies from neuron to neuron, as does the amplitude and waveform of the recorded action potentials. In our laboratory we have adopted the convention of calling the olfactory receptor neuron which produces the largest amplitude spike the A cell, that producing the next largest, the B cell and so on. Occasionally a very large amplitude spike is observed which is assumed to arise from a mechanoreceptor located at the base of the sensillum. Figure 2 shows typical extracellular recordings obtained from trichodeal receptor neurons on the antenna of the male redbanded leafroller moth following stimulation with several intensities of the two 14 : AC isomers which are important components of the females pheromone blend. Note in particular that both of the receptor neurons in the sensillum respond to both Z and E11–14 : AC but that they differ greatly in their relative sensitivity to these materials. Since both neurons are immersed in the same microenvironment within the sensillum we must assume

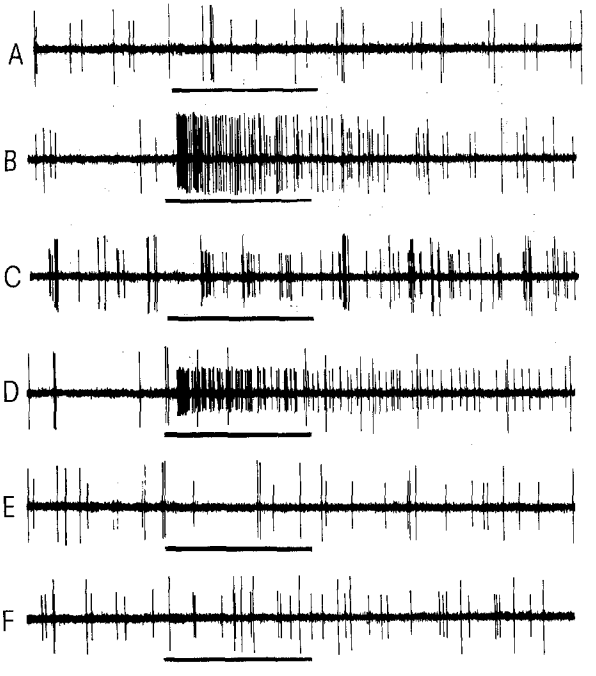

Figure 2. Extracellular recordings from a single trichodeal sensillum on the antennae of a male RBLR showing responses to measured doses of the three identified female pheromone components. The scale bar below each record indicates the application of a 2-s puff of pheromone from an odor cartridge containing the indicated amount of compound. A) 0.32 μg Z11–14:AC; B) 32 μg Z11–14:AC; C) 0.32 μg E11–14:AC; D) 32 μg E11–14:AC; E) 316 μg 12:AC; F) 1 μl mineral oil.

that these differences in response characteristics are intrinsic properties of the neurons and not some accidental result of the mode of stimulus presentation.

At this point I should reiterate one of the basic tenets of neurophysiology concerning the role of action potentials in the transmission of information within the nervous system. For any one neuron, action potentials are generally all or none, that is, they occur or they do not. Each one that does occur is indistinguishable from any other. Therefore action potentials do not carry labels which identify the process which caused them to be elicited. Consequently once an action potential occurs, the identity of the stimulatory event, if any, is lost. Because of this fact there are only four general aspects of neural discharge available for the encoding of environmental information. These aspects are exemplified in the following hierarchical list of questions which one may ask about the response properties of olfactory receptor neurons. 1) Was an action potential generated in the neuron or not? 2) What was the distribution of responding neurons for a particular stimulus intensity among all of the potentially active neurons? 3) What was the temporal distribution of action potentials in any one neuron? and 4) How did this temporal pattern vary across all of the potentially active neurons? It should be clear from an examination of figures 2–4 that a range of different stimuli or stimulus intensities can elicit qualitatively and quantitatively similar amounts of activity in a particular receptor neuron. Thus odor identification is a challenging problem in neural encoding.

The response measures one chooses to make following stimulation reflects in large measure one's underlying assumptions about the nature of the encoding process in olfactory receptor neurons. The measures most often found in the literature range from those in which responses to stimuli are simply enumerated as excitatory, inhibitory or without effect[30], through those which accumulate individual action potentials within some arbitrary length of time[12, 32], to those which address the temporal pattern of action potential discharge in individual neurons[11, 26]. Although there is nothing inherently "correct" about any particular index of activity it should be apparent that the simplest level of measurement which allows one to predict behavioral outflow given knowledge of the electrophysiological efficacy of a compound is the most logical starting point. For example, there would be little reason to specify the temporal pattern of discharge in a neural system in which all known stimuli map uniquely onto two mutually exclusive behaviors. If every compound which excites the olfactory receptor neurons under study elicits one common behavioral response and every compound which fails to elicit that behavior also fails to excite neural activity in the same set of receptors, then the simple presence of activity in the particular neurons may serve to code for the behavioral response. However one should recognize that one of the signal features of any sensory receptor neuron it its ability to transform environmental events into both spatial, that is which neurons are active, and temporal patterns of neural activity. Therefore as the complexity of the chemical communication system increases, it is likely that spatial and temporal patterns of discharge will become increasingly important in the encoding process simply because they are capable of encoding more information than simpler schemes[32, 36–38]. In addition one should not lose sight of the fact that several chemosensory receptor neurons are often packaged together within a single sensillum. These neurons are often sister cells developmentally and share the same extracellular space. Consequently measures of neural discharge which compare the electrical activity elicited simultaneously in each of the receptor neurons within a sensillum are likely to be important. In a like manner we should note that in many instances behavioral responses to chemical stimulation occur within a few hundred milliseconds of stimulus application, at which time only a few action potentials have been elicited. Therefore when considering various measures of neural activity, especially those involving temporal patterns of discharge, only those portions which occur within the behavioral response time need be evaluated[11].

C) Chemical receptor electrophysiology

Progress towards understanding the receptor neuron mechanisms underlying the perception of sex attractant pheromones is largely due to the pioneering efforts of Schneider and his colleagues with the domestic silk worm moth *Bombyx mori*[18]. These studies initially gave rise to the two paradigmatic descriptions of olfactory receptor neuron specificity; that is, odor specialists and odor generalists. Extracellular microelectrode recordings obtained from the olfactory receptor neurons within the sensilla on the male antenna have shown that the synthesized sex attractant pheromone, Bombykol (E, Z-10, 12-hexadecadienyl alcohol), and several of its related isomers excite the receptor neurons in only the sensilla trichodea. The quantitative and qualitative uniformity of their specificity to the pheromone led Schneider to call the neurons contained within these sensilla, odor specialists. In contrast, odor generalists are receptor neurons found in the short sensilla basaconica which respond to a wide array of nonpheromone odors. No two of these generalists seem to have exactly the same pattern of responsiveness although each response spectrum remains stable over a period of time. Moreover the response spectra of generalists and specialists are presumed not to overlap. It was long assumed that the afferent outflow from *Bombyx* odor specialists was linked directly to the motor mechanisms controlling sex behavior, with little, if any, intervening central processing because whenever the specialist was activated, even by certain nonpheromone compounds, sexual behavior would result. Therefore the only important question for coding in *Bombyx* was: are the trichodeal receptor neurons activated? An additional few hundred action potentials in the whole population of specialists was thought to be sufficient to elicit a full blown sexual response on the part of the male.

The generalist receptor neurons with their broad range of sensitivity produce a large number of different spatial and temporal patterns of neural discharge. These patterns are thought to undergo extensive central processing which, in some as yet unknown way, results in the behavioral response characteristic of the stimulating compound.

As work on the pheromone receptor neurons of *Bombyx* and of other species has progressed, there has arisen a

growing indication that the sharp dichotomy between generalists and specialists as originally described for *Bombyx* may be too simple. Recent studies by Kaissling and his colleagues[19] have revealed that the pheromone communication system of *Bombyx* is relatively complicated, with calling females producing at least three related compounds. The first is the previously identified diunsaturated alcohol, Bomkykol, the second is the E, E isomer of Bombykol and the third is Bombykal, the aldehyde of Bombykol. In these studies the B cell of the long trichodeal sensillum responds preferentially to Bombykal while the A cell response preferentially to Bombykol. However, large amounts of Bombykal may activate the A cell along with the B cell. Moreover there isn't any obvious interaction in the response properties of the two receptor neurons when both compounds are presented simultaneously, as is done by a calling female. Behavioral observations of male sex behavior when Bombykol and Bombykal are presented alone and in combination, reveal that both low doses of Bombykol and high doses of Bombykal elicit sex behavior. Since both of these treatments also excites the A cells of a sensillum one could continue to assume that activity in these neurons is both necessary and sufficient for eliciting sex behavior and that little central processing is in fact required. However when mixtures of the two compounds are evaluated simultaneously, the addition of even a small amount of aldehyde to Bombykol completely eliminates behavior, in spite of the fact that the A cell is activated in the normal fashion. Consequently the outflow from the A cell in conjunction with that from the B cell must be involved in the central decision process which gives rise to sex behavior. Moreover, since sex behavior can be elicited in situations where A cell activity exceeds B cell activity and vice versa, some feature of the neural discharge in the two cells, other than bulk activity, must convey the necessary information for the behavioral response.

Additional levels of complexity in both the pheromone communication system and in electrophysiological response properties are obtained in the redbanded leafroller moth. As noted earlier, the female of this species produces at least 3 pheromone components which collectively are powerfully attractive to males. As shown in figure 2, both A and B receptor neurons in a single sensillum respond to the (Z) and (E) components of the pheromone blend[31,32]. Dodecyl acetate is relatively ineffective in both neurons. On the average the A receptor neuron is more responsive to Z11–14:AC while the B neuron is more sensitive to E11–14:AC for most of the intensity range so far examined. However, unlike the situation reported for *Bombyx* there is considerable quantitative variation among the sampled receptor neurons for these two compounds. These variations are most easily appreciated by examination of figure 3 which details individual dose response functions for the A and B receptor neurons found in more than 20 different trichodeal sensilla when stimulated with graded intensities of Z11–14:AC. Companion data obtained with graded intensities of E11–14:AC are shown in figure 4. There is clearly a range of different stimulus intensities within which two behaviorally discriminable compounds can elicit the same absolute discharge magnitude in a given receptor neuron. Obviously then, absolute discharge magnitude in any particular receptor neuron cannot code for odor quality because this neural measure is always confounded by odor intensity. Odor quality may be unambiguously encoded if we consider the relative amount of electrical activity elicited in the whole ensemble of A and B olfactory receptor neurons. In more than 80% of the sensilla examined Z11–14:AC elicited larger responses in the A receptor neuron when compared to the responses elicited simultaneously in the B neuron. This relative relationship held over the range of stimulus intensities examined. The opposite relation was obtained when these same neurons were stimulated with E11–14:AC. That is, the response of the B neuron was usually larger than the response of the A neuron.

It should be apparent that these two components of the RBLR pheromone blend could be simply encoded by a

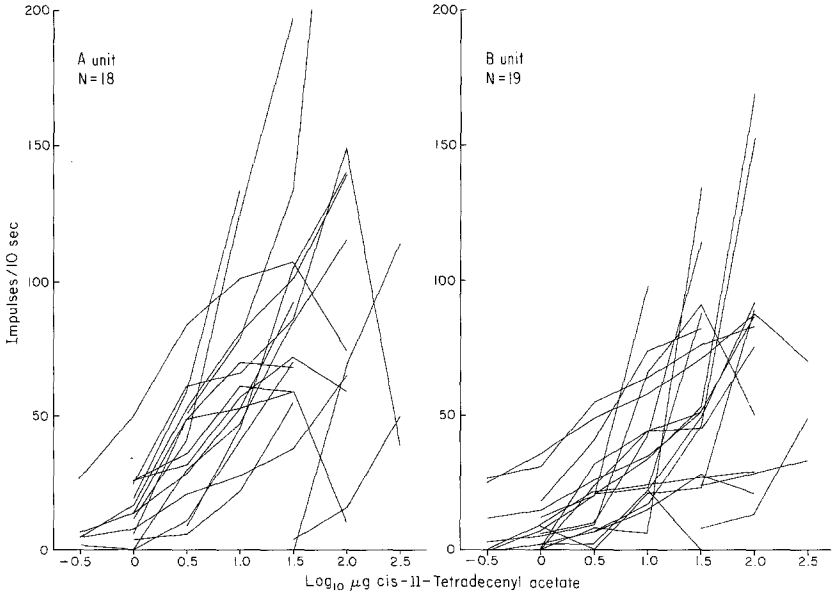

Figure 3. Response functions for 18 A and 19 B pheromone receptor neurons. These include 17 pairs of receptor neurons each obtained from a separate sensillum. The abscissa is the number of µg of Z11–14:AC loaded into the stimulus cartridge and not the amounts actually arriving at the receptor neurons.

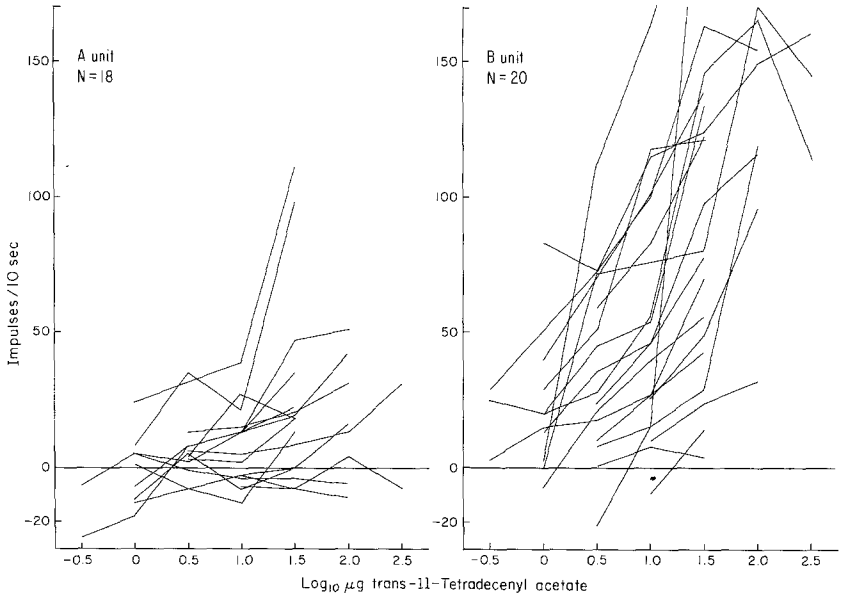

Figure 4. Response functions for 18 A and 20 B pheromone receptor neurons, including the 17 pairs of receptor neurons shown in figure 3 when stimulated with E11–14:AC. Responses which are less than 0 impulses/10 s are those in which the level of spontaneous activity in the 10-s period before stimulation was higher than the driven activity observed in the subsequent 10-s period.

central process which compares the relative pattern of activity across the whole sample of receptor neurons. The quantitative variation observed in the sample of single receptor neurons would serve both to enhance the microstructure of the across neuron pattern of activity, and to allow for encoding the intensive aspects of the stimuli[32, 34]. As noted earlier, most of the pheromones that we have considered are blends of several individual compounds. In some species the relative amounts of two compounds may be important whereas in others it may be the composition of the total blend that modulates behavior[2, 24]. Therefore it is important for our understanding of neural encoding to investigate the response porperties of pheromone receptor neurons when stimulated with blends and to contrast these responses to those obtained with the single components of the blend. Mixtures which contained 12:AC were initially of interest, in both RBLR and CL, because this compound is known to modulate behavior when presented with primary components of the pheromone, but is rarely an effective stimulus electrophysiologically when used alone[32, 36]. Although it is possible that there are highly specific receptor neurons for 12:AC elsewhere on the animal, we sought to evaluate its effects when presented with one or the other of the components of the RBLR pheromone (fig. 5). Each observed response shows significant deviation from that expected from the simple sum of the two single component stimuli. This was especially clear in the response to the mixture which included 12:AC. Although this single compound did not increase action potential production when used alone, it greatly enhanced both the peak instantaneous frequency and the magnitude of the tonic level following stimulation when mixed with Z11–14:AC. The same A neuron showed reduced responses to a 1:1 mixture of the two 14:AC isomers when they were applied simultaneously. Hence, unlike the situation in *Bombyx*, mixtures of stimuli can differentially modify the response properties of individual sensory neurons. Moreover a single receptor neuron may show non-linearities in either direction, depending on the composition of the mixture used as a stimulus. Although there is individual variability among receptor neurons, enhanced responses to mixtures containing 12:AC averaged 145% of that expected, whereas responses to mixtures containing E11–14:AC were reduced an average of 75%[31]. These preliminary mixture experiments show that individual pheromone receptor neurons may interact with certain components of the pheromone blend, not to directly alter the cells' action potential production but, in a way which changes the efficacy of other components of the blend to influence electrical activity.

Finally, it should be noted that an individual species may have more than one type of pheromone receptor neuron. These types may be characterized by the spectrum of compounds which are effective stimuli or by the intensity

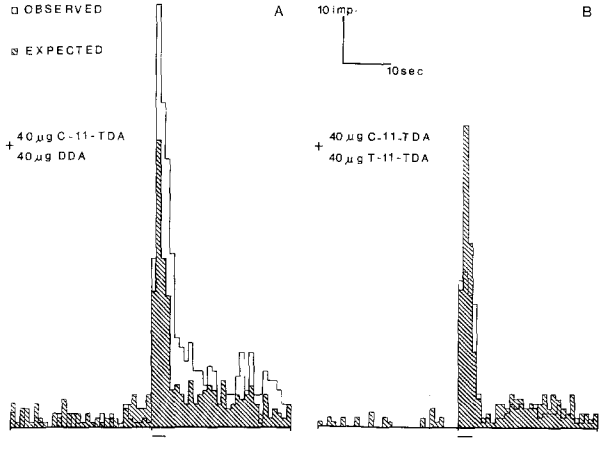

Figure 5. Event-time histograms of the responses of one A receptor neuron in a male RBLR sensillum trichodea. *A* Observed response to a 1:1 mixture of Z11–14:AC (C-11-TDA) and 12:AC (DDA) each at 40 µg. *B* Observed response to a 1:1 mixture of Z11–14:AC and E11–14:AC (T-11-TDA) in the same neuron. Each observed response (open bars) plots the number of action potentials which occurred in successive 1-s time bins. The bar under each response indicates the 3-s stimulus application. The expected response plot (hatched bars) was obtained by adding together graphically the event-time histograms obtained from each of the single component stimuli. For this neuron only Z11–14:AC was an effective single component stimulus.

range within which they operate[36]. The complexities outlined in the peripheral olfactory system are likely to be further modified and transformed by the capabilities of the central olfactory system in ways yet to be fully described.

Conclusions

In conclusion, olfactory pheromones are widespread in nature and control many fundamental aspects of an insect's life. Detailed behavioral observations in the field and laboratory indicate the great complexity of most pheromone communication systems. The preliminary electrophysiological experiments on single olfactory receptor neurons that we have described here, point toward the possibility of unraveling the basic physiological mechanisms underlying these behavioral complexities. This information is directly applicable to many problem areas in chemoreception and could provide a base for the rational use of pheromones in the control of insect pests.

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Bacterial chemotaxis and vertebrate olfaction

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Key words. Olfaction; bacterial chemotaxis; stimulus; receptors; transduction; response assay.

Our understanding of the biochemistry of vertebrate olfaction is very limited. Since so many biochemical systems have been conserved across evolution, we might hope to learn something about olfaction by examining the chemical recognition systems of less advanced organisms. Bacterial chemotaxis is one such system that is understood in some detail.

It is my intention here to assess bacterial chemotaxis and vertebrate olfaction from as common a perspective as is possible. The discussion is restricted to those properties where comparisons between the two systems can be made. Chemoreception and initial events in stimulus transduction offer the greatest possibilities in this regard. In a sense I will be trying to compare single-celled bacteria with single olfactory receptor neurons. Olfaction could also be related to chemotaxis in slime molds, protozoans, various invertebrates, leukocytes, and so forth, but I have chosen to make a detailed comparison with the bacterial system.

Even though the discussion of chemotaxis has been confined to two species of bacteria, it is far from complete. There exist many more comprehensive reviews[5,61,62,74,75,84,124]. For other reviews of vertebrate olfactory reception, see Lancet[80] or Cagan and Kare[24].

Bacterial chemotaxis

1. The organisms

The most enlightening studies of bacterial chemotaxis have been done with two closely related species of enteric bacteria, *Escherichia coli* and *Salmonella typhimurium*. The single cell of either organism is rod-shaped, about 1 μm in diameter and 1–2 μm long. From each cell protrude 6–8 flagella, each of 5–10 μm length. Certain properties of these organisms have contributed greatly to their successful study. Generation times in liquid culture are commonly 0.3–2 h, with 2 ml of growth medium yielding 10^9 cells. Results from such cultures may be treated as averages of enormous numbers of identical single organisms. Highly developed methods for selecting mutants and transferring their genetic defects have resulted in collections of strains with widely varying chemotactic defects. The experimenter can manipulate the extracellular environment at will by centrifuging the bacteria and resuspending them in a new medium. The cells are not fragile, and the rights of experimental bacteria are rarely called into question.

The involvement of transmembrane potential is difficult to study in such small cells. Pharmacologically produced giant *E. coli* cells can be penetrated with intracellular microelectrodes[40], but this method has not yet been applied to the study of chemotaxis.

2. Assays of response

Microscopy has enabled description of the behavioral responses of single bacteria to chemical stimuli. In an unchanging environment, the cell alternates at random between two swimming states[18,85]. In one state, the several flagella, rotating counterclockwise[81], work together as a bundle to propel the cell smoothly ahead[16,17,121]. In the other state, the flagella rotate clockwise[81]. Due to the helical sense of the flagella, this causes the bundle to fly apart[86,87], and the cell 'tumbles' in place. When smooth swimming resumes, it will be in a new, randomly chosen direction[18]. The cell is able to migrate toward or away from the source of a chemical by modulating the proportion of time it spends in each state. If an episode of smooth swimming is bringing the cell closer to the source of an attractant, smooth swimming is prolonged (i.e. tumbling is suppressed)[18,85]. If the cell is going away from the attractant, it will soon tumble[85] and try swimming in a new direction. The responses to attractants and repellents are opposite, and so the cell can also migrate away from a repellent source[134]. It is able to integrate simultaneous stimuli[134,135], although the response is not always the exact