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2 Neurobiology of Pheromone Perception*

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1 INTRODUCTION

Pheromone neurobiology is a relatively new discipline in the biological sciences because its inception required the development of a broad, detailed foundation of knowledge from neurophysiology, physiology, chemistry, animal behavior, and physics. The multidisciplinary nature of this field imposes serious difficulties on attempts to synthesize new concepts, to conduct useful research, and to apply knowledge to practical ends. The beginnings of pheromone neurobiology can be traced back only some 25 years. At that time, magically small quantities of pheromones appeared to elicit behavior from kilometer distances. Nowadays the attraction distances and the threshold pheromone concentrations are more realistic albeit less spectacular.

2 CONCEPTS AND PERSPECTIVES

Many of the original concepts of pheromone neurobiology come from studies of *Bombyx mori* L. because it was from this insect that a pheromone was first chemically identified. Preceding the final identification by Adolph Butenandt were the astute descriptive studies of pheromone-induced behavior by Ilse Schwink. During and following the identification, Dietrich Schneider and his colleagues were setting the foundations for all subsequent neurobiological studies. We nonetheless believe that some of the concepts based on studies of *B. mori* are difficult to extend unaltered to other species.

The aim of this chapter is to provide a critical overview of the subject from a combined biological and physical perspective. We discuss the natural progression of events that occur following pheromone synthesis and release, i.e., the transduction and perception of pheromone, the central processes that control discriminatory behavior, and some of the inferences that can be made from behavioral responses.

3 SYNTHESIS AND RELEASE OF PHEROMONE

The glandular cells and organs that synthesize and release pheromones into the environment occur in many locations on or in the body of female as well as male insects. In most Lepidoptera, portions of the release process, e.g., extrusion of the pheromone gland, are probably under direct neural control although nothing is known about these mechanisms (Conner, W. *et al.*, 1980). The morphological and biosynthetic diversity of these cells and organs is known in detail for only a few species. Very little effort has been extended toward studies of pheromone biosynthesis. In the well-documented cases of *Trichoplusia ni* (Hübner), *Galleria mellonella* (L.), and *Argyrotaenia velutinana* (Walker), *de novo* synthesis of pheromone precursor fatty acyl moieties occurs from acetate (Schmidt, S. and Monroe, R. 1976; Jones, I. and Berger, R. 1978; Bjostad, L. *et al.*, 1981). Presumably, the olefin chain is synthesized via normal lipid two-carbon condensations and not from externally derived fatty acid analogs of pheromone (Bjostad, L. and Roelofs, W. 1981). More specialized enzymes must exist to convert the precursors to the particular pheromones. In the case of bombykol [(*E,Z*)-10,12-hexadecadien-l-ol] the precursor acid is dehydrogenated (Inoue, S. and Hamamura, Y. 1972; Yamaoka, R. and Hayashiya, K. 1982), whereas the olefin of disparlure [(7*R*,8*S*)-*cis*-7,8-epoxy-2-methyloctadecane] is epoxidized (Kasang, G. *et al.*, 1974).

Because almost nothing is known about the neural control of biosynthesis, in this section we will deal primarily with the neural and/or hormonal control of calling behavior and pheromone release. Also, we will consider some of the physics of atmospheric dispersal of pheromone.

3.1 Neural control of calling behavior and pheromone release

At this time it is not certain whether calling behavior is induced by releaser hormones (see section 6.2.3) or by some other more direct neural control. According to Riddiford, L. and Williams, C. (1971), a neurohormone from intact corpora cardiaca releases calling behavior in *Antheraea polyphemus* (Cramer). They found the photoperiod to be more instrumental in initiating calling behavior for *Hyalophora cecropia* (L.) while *Bombyx mori* and *Antheraea pernyi* Guérin were continuously attractive. Further experiments by Riddiford, L. (1974) suggested that hormonal induction of calling required exposure to (*E*)-2-hexenal (Riddiford, L. 1967). However, there is controversy about the linkage between calling behavior of *A. polyphemus* and exposure to (*E*)-2-hexenal (Cardé, R. and Webster, R. 1980). Demonstrating the generality of a releaser hormone effect for this behavior will require further experiments.

Most of the experimental efforts on other insects point instead to a more direct neural control of the onset of calling behavior. A detailed account of exogenous factors affecting female release of pheromone is given by Cardé, R. and Webster, R. (1980). They identify a number of factors affecting the release of calling behavior and pheromones by females. Light is the predominant factor. It sets the biological clock which times calling behavior and pheromone release and thus co-ordinates male activity. Temperature, humidity, and wind speed also have been demonstrated to affect female calling behavior, and certainly some of the peripheral receptor cells monitoring these environmental factors are found on the antenna. However, deantennated females of *A. polyphemus* were as attractive as females with antennae (Cardé, R. and Webster, R. 1980). Thus, the primary factor governing the onset of calling behavior must be light. Light could act directly on the endogenous clock itself, but if perceived through the eyes it could affect behavior more directly through the major coordination centers of the brain.

The modifier effects of hormones on both calling behavior and pheromone synthesis have been examined in detail by Truman, J. and Riddiford, L. (1974, 1977). Although the pertinent hormones are elaborated and stored within brain cells—and, indirectly, probably modify neural responses—a review here is outside the scope of this chapter.

There have been few investigations exploring the direct neural control of pheromone emission. The spotting of trail-following pheromones by termites and ants appears to be under neural control. Abdominal movements associated with pheromone release by *Lymantria dispar* L. females were not eliminated by resection of the ventral nerve cord anterior to the terminal abdominal ganglion. Although brain removal just prior to eclosion did not affect the daily rhythm of movements, it eliminated the response of males either by affecting the synthesis or by the release of pheromone (Hollander, A. and Yin, C. 1982). The pulsatile signalling noted in the emission of pheromones by *Utetheisa ornatrix* (L.) (Conner, W. *et al.*, 1980) and by *T. ni* (Bjostad, L. *et al.*, 1980a) also implies some active role of the nervous system. It is unlikely that this pulsating release is important for information transfer over more than a few centimeters (Bossert, W. 1968; Mankin, R. *et al.*, 1980a). Because little information transfer is likely, we consider the pulsation to be a reflection of alternate relaxations of the hydrostatic pressure necessary for gland extrusion that are possibly under ganglionic control. Because less pheromone is released than when the gland of *Trichoplusia ni* is forcibly and constantly extruded (Bjostad, L. *et al.*, 1980a), pulsation may conserve the supply of pheromone and prolong the period of release of pheromone during calling. Conner, W. *et al.* (1980) consider other alternatives that we consider less likely.

3.2 Release rates and volatility of pheromone

The release of pheromone from a gland is a poorly understood evaporation process. Major factors affecting the release are the vapor pressure and diffusion coefficient of the sex pheromone, the size and form of the pheromone gland, the interactive forces

Table 1: *Pheromone gland contents and release rates for several insects*

Insect	Pheromone gland contents	Approximate quantity released (ng/insect per min)	Source
Ephestia cautella	(1) (*Z,E*)-9,12-tetradecadien-1-ol acetate	0.22	Coffelt *et al.* (unpublished)
	(2) (*Z*)-9-tetradecen-1-ol acetate	0.04	
Grapholita molesta	(1) (*Z*)-8-dodecen-1-ol acetate	0.5	Baker, T. *et al.* (1980)
	(2) (*Z*)-8-dodecen-1-ol*	0.11	
Lymantria dispar	*cis*-7,8-epoxy-2-methyloctadecane	0.16–29	Richerson, J. and Cameron, E. (1974)
Plodia interpunctella	(1) (*Z,E*)-9,12-tetradecadien-1-ol acetate	0.05–0.07	Nordlund, D. and Brady, U. (1974)
	(2) (*Z,E*)-9,12-tetradecadien-1-ol	0.10	Sower, L. and Fish, J. (1975)
Trichoplusia ni	(1)(*Z*)-7-dodecen-1-ol acetate	18 (12–22)	Bjostad, L. (1978); Bjostad, L. *et al.* (1980a);
	(2) dodecan-1-ol acetate	1.3	Szentesi, Ä. *et al.* (1977)
Dendroctonus brevicomis	(1) 3-methylene-7-methyl-1,6-octadiene (myrcene)	40 (SE ± 9.7)	Browne, L. *et al.* (1979)
	(2) *exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane (*exo*-brevicomin)	0.65 (SE ± .08)	
	(3) *endo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane (*endo*-brevicomin)	0.2 (SE ± .07)	
	(4) (*E*)-(+)-2,6,6-trimethylbicyclo[3.1.1]hept-2-en-4-ol (*trans*-verbenol)	1.7 (SE ± .25)	
	(5) 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-en-4-one	0.006 (SE ± 0.002)	
Trogoderma variabile	(1) (*Z*)-14-methyl-8-hexadecen-1-al	0.025	Greenblatt, R. *et al.* (1977)
	(2) (*Z*)-14-methyl-8-hexadecen-1-ol	0.000028	
	(3) methyl 14-methyl-8-hexadecenoate	0.000021	
	(4) γ-caprolactone	0.010	
	(5) methyl 7-hexadecenoate	0.000063	
Trogoderma inclusum	(1) (*Z*)-14-methyl 8-hexadecen-l-al	0.0014	Greenblatt, R. *et al.* (1977)
	(2) (*Z*)-14-methyl-hexadecen-1-ol	0.000021	
	(3) methyl (*Z*)-14-methyl-8-hexadecenoate	0.00027	
	(4) methyl 7-hexadecenoate	$< 10^{-5}$	
Trogoderma granarium	(1) (*Z*)- and (*E*)-14-methyl-8-hexadecen-1-al	0.0010	Greenblatt, R. *et al.* (1977)
	(2) 14-methyl-8-hexadecen-1-ol	0.0000069	
	(3) methyl (*E*)-14-methyl-8-hexadecenoate	0.000014	
	(4) hexanoic acid	0.0021	
	(5) γ-caprolactone	0.0013	
	(6) methyl 7-hexadecenoate	0.0000069	
Trogoderma glabrum	(1) (*E*)-14-methyl-8-hexadecen-l-al	0.019	Greenblatt, R. *et al.* (1977)
	(2) (*E*)-14-methyl-8-hexadecen-l-ol	0.000014	
	(3) methyl (*E*)-14-methyl-8-hexadecenoate	0.000014	
	(4) hexanoic acid	0.0065	

Table 1: Continued

Insect	Pheromone gland contents	Approximate quantity released (ng/insect per min)	Source
	(5) γ-caprolactone	0.0054	
	(6) methyl (*Z*)-7-hexadecenoate	0.000022	
Scolytus multistriatus	(1) (−)-4-methyl-3-heptanol	0.0080	Gore, W. *et al.* (1977)
	(2) (−)-α-multistriatin	0.0092	
	(3) (−)-α-cubebene	0.057	

* Ratios of minor components identified by Cardé, R. *et al.* (1979).

between the pheromone molecules and the other chemical constituents of the cuticle over the gland, and environmental factors such as wind speed, turbulence, and relative humidity (Regnier, F. and Goodwin, M. 1977; Hirooka, Y. and Suwanai, M. 1978; Mankin, R. *et al.*, 1980a). Saturated vapor pressures of several sex pheromones and closely related compounds are listed in Hirooka, Y. and Suwanai, M. (1978). In general, the saturated vapor pressure of 12-16-carbon sex pheromones is about 1×10^{-4} cmHg, and the diffusion coefficient of a sex pheromone is about 0.05 $cm^2\ s^{-1}$ (Mankin, R. *et al.*, 1980a).

Table 1 lists the pheromone gland release rates for the insects that have been studied to date. Both the quantity in the gland and the release rates vary considerably among insects. Indeed, it has been shown that a chemical which can inhibit the response to pheromone is present in the gland of some Lepidoptera but is not released in detectable quantities. By contrast, it has been shown that the most active component of the pheromone of *Trogoderma variabile* Baillon, *Trogoderma inclusum* LeC., *Trogoderma granarium* Everts, and *Trogoderma glabrum* Herbst, (*Z*)- and/or (*E*)-14-methyl-8-hexadecenal, absent from the beetle in detectable quantities, is the component released in the greatest quantity (Greenblatt, R. *et al.*, 1977).

3.3 Atmospheric dispersal processes

The molecular diffusion coefficient of pheromone is so small that, except in still air, the primary modes of dispersal are convective and turbulent diffusion. At present, both convective and turbulent diffusion can be described only in terms of smoothed statistical averages and standard deviations, usually taken over 3-min or longer periods of time (Mankin, R. *et al.*, 1980a). Consequently, although several mathematical models of pheromone dispersal have been proposed, their applicability to determining the instantaneous concentration of pheromone is questionable. To the extent that such models are valid, we make the following generalizations.

After pheromone is released from a gland it is carried convectively in compact filaments. Turbulent eddies of the same size as a filament thickness tend to spread the filament quickly. Eddies either much smaller or larger than the filament thickness have little effect on the spread of the filament, they only transport pheromone within the filament or move the filament as a whole. When a puff of pheromone is released, it first spreads slowly because the width of a filament is smaller than the smallest turbulent eddies ($\sim$ 5–10 cm). This critical width is reached within 0.5–10 m downwind, where the puff splits into several filaments and begins to spread rapidly, eventually becoming indistinct from the background. The greater the wind speed, the faster the pheromone filaments disperse.

Searching insects may utilize the changing nature of the pheromone plume to help locate pheromone sources. Far from the source the plume is indistinct and relatively uniform. Close to the source the plume is discontinuous, irregular, and tightly defined. The insect in its casting motions can easily detect the resultant irregular temporal modulation of the olfactory signal, as has been shown by electroantennogram (EAG) measurement (Conner, W. *et al.*, 1980). This topic is discussed in more detail in Mankin, R. *et al.* (1980a) and in section 6.2.1.

4 PERCEPTION OF PHEROMONE

A complex sequence of events must occur for a pheromone to elicit a meaningful behavioral response from an animal. First, the airborne

pheromone molecules must adsorb to one of an ensemble of receptor end-organs and depolarize a receptor neuron. The action potentials initiated by this depolarization then travel to the deutocerebrum where the responses from the entire ensemble of neurons are integrated. Further processing and the integration of olfactory inputs with other sensory modalities occur in the mushroom bodies, the protocerebral lobes, and the suboesophageal ganglion before an observable behavioral response is effected. We will discuss the movements of pheromone molecules from the air to dendrites of olfactory receptor cells, transduction, the connections of receptor cells with the CNS and the quantification of the stimulus information at these different stages of perception.

Although pheromones usually consist of blends of more than one chemical entity, in this section, for simplicity, we will consider a pheromone to be a single chemical. Later sections will discuss the discrimination of different blends at the sensory periphery and at the behavioral level.

4.1 Deposition of pheromone to the antenna and transport to site of action

The series of events culminating in pheromonal perception begins when the pheromone adsorbs to the

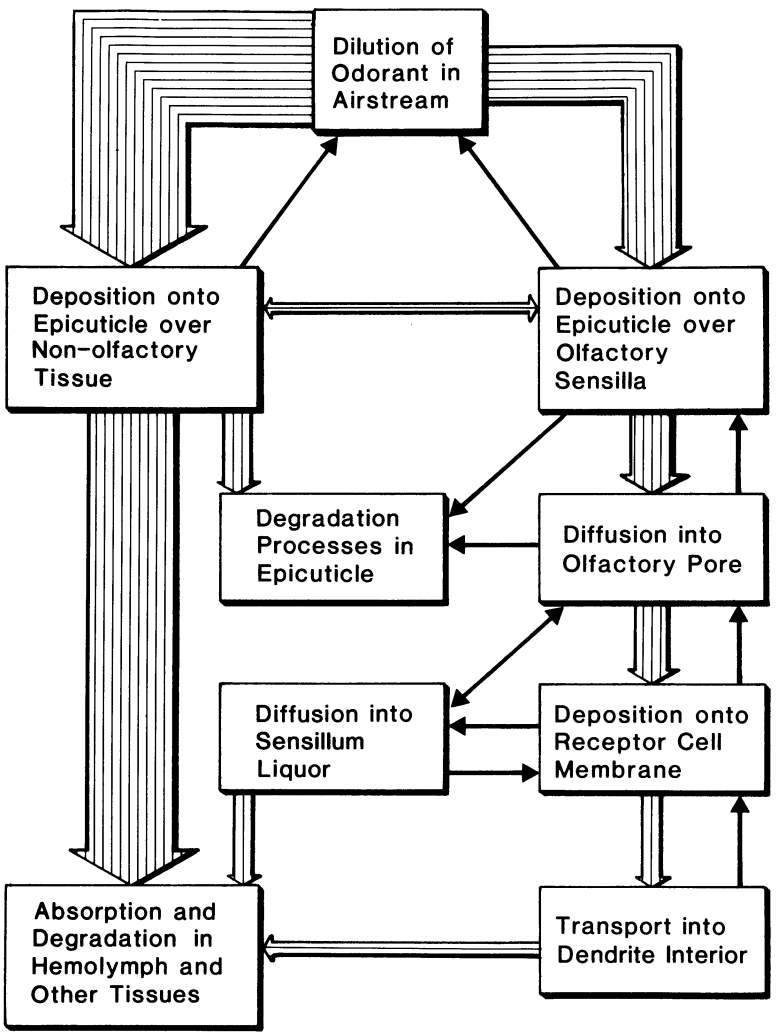

FIG. 1. Hypothetical pathway for pheromone deposition, diffusion, and degradation on and within the insect olfactory sensillum. The width of the arrow corresponds to the expected fraction of the stimulus following a particular path.

antennal epicuticle (Fig. 1; also see Kasang, G. 1973). There is some controversy whether the pheromone adsorbs preferentially to the cuticle of long, thin olfactory hairs called sensilla (Fig. 2), or whether it adsorbs uniformly to all parts of the antenna. Adam, G. and Delbrück, M. (1968) and Murray, J. (1977) assumed that all the pheromone molecules impinging onto the cuticle are captured, i.e., the cuticle is a perfect sink. If this were true, the shape of the sensilla would enhance the difference between the pheromone concentration in the air a few nanometers away from the sensillar surface and the concentration in the air a few micrometers away (Kaissling, K. 1971). Because of the induced concentration gradient, the sensillum would collect about four times as many molecules per unit surface area as the main flagellum (Steinbrecht, R. and Kasang, G. 1971). However, the predicted rate of

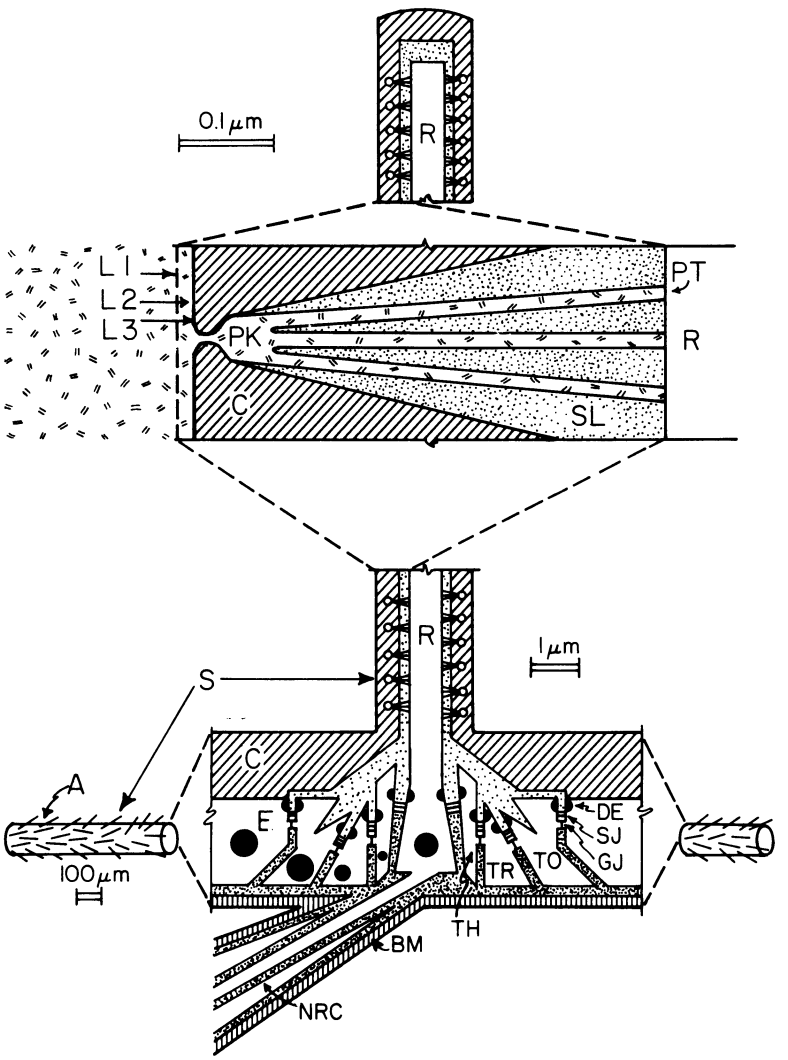

FIG. 2. Scaled representation of a lepidopteran pheromone receptive sensillum. One or more receptor cells are surrounded respectively by the thecogen, trichogen, and tormogen cells. Desmosomes and septate junctions electrically isolate the sensillum liquor from the hemolymph, and gap junctions electrically interconnect all cells except the receptor cells. Nomenclature: A = antenna; BM = basement membrane; C = cuticle; DE = desmosome; E = epidermal cell; GJ = gap junction; L1, L2, L3 = layers of the epicuticle; NRC = neurilemma cell; PK = pore kettle or channel (more like a channel in pheromone-sensitive sensilla); PT = a pore tubule; R = the receptor cell; S = sensillum; SJ = septate junction; SL = sensillum liquor; TH = thecogen cell; TO = tormogen cell; TR = trichogen cell.

deposition, based on the assumption that the cuticle is a perfect sink, is a factor of 10 higher than the rates actually observed by Mankin and Mayer in as yet unpublished work. Therefore, it is more realistic to assume that most of the impinging pheromone molecules are re-entrained into the air (see Judeikis, H. and Stewart, T. 1976). This finding leads to a different prediction: the rate of deposition is directly proportional to the exposed surface area and is essentially independent of the air velocity or the shape of the flagellum and sensilla. The pheromone should therefore deposit uniformly to all parts of the antenna, excepting minor variations due to roughness.

Whichever the pattern of deposition, only a fraction of the deposited pheromone molecules actually are involved in the perception process. For Lepidoptera, this is primarily the fraction that adsorbs to the long, tapered sensilla trichodea innervated by pheromone-sensitive olfactory receptor cells. The molecules that adsorb to other parts of the antenna are probably degraded by enzymes in the cuticle, hemolymph, and other tissues. Tritiated bombykol is changed into an acid and an ester on the antenna of *Bombyx mori* after uptake (Kasang, G. 1971, 1974). Kasang, G. and Kaissling, K. (1971) and Kaissling, K. (1974) concluded that these reactions of bombykol are unspecific "secondary processes" that presumably would remove active molecules from the receptor site after the stimulus ended. This conclusion was based on measurements that indicated "saturation" of the degradative process occurred above 10^{13} molecules per antenna, while the generator potentials (measured by the EAG) became saturated at about 3×10^{11} molecules per antenna. Esterases that degrade acetate pheromones are found on the antenna of *Trichoplusia ni* (Ferkovich, S. *et al.*, 1973), *Manduca sexta* (L.) and *Antheraea polyphemus* (Vogt, R. and Riddiford, L., 1981). The esterases in *T. ni* appear to be located in membranes isolated from olfactory sensilla (Mayer, M. *et al.*, 1976) and appear to be specific for the pheromone *in vivo* (Mayer, M. 1975) and *in vitro* (Ferkovich, S. *et al.*, 1980).

Once a pheromone molecule is deposited onto a sensillum trichodeum, it diffuses two-dimensionally along the epicuticle. The path to its final destination is suggested by the sensillar structure and composition, which we will now review briefly. The sensillar epicuticle is not a simple layer of wax, but instead comprises three distinguishable regions designated by Steinbrecht, R. and Kasang, G. (1971) and Steinbrecht, R. (1973) as L1, L2, and L3 (Fig. 2). The L1 layer is faint, only about 2.5 nm thick, and its consideration as an actual layer is based on its presumed protection of the L2 layer against extraction by chloroform-methanol (Filshie, B. 1970, 1982; Steinbrecht, R. and Kasang, G. 1971). Hawke, S. (1970) also observed apparent differences in extractability of L2 by both acetone and chloroform, although incubation of the antenna in pronase failed to affect L2. No observations of L1 were made; thus, there appears to be some uncertainty to the existence of L1 as an actual layer. Steinbrecht, R. and Kasang, G. (1971) consider L1 and L2 to be a surface coat of polymeric lipids.

The staining properties of the L1 layer suggest that it may be partly proteinaceous and that it or L3 may be the site of enzymes shown to degrade the pheromones (Kasang, G. 1974; Ferkovich, S. and Mayer, M. 1975; Vogt, R. and Riddiford, L. 1981). Locke, M. (1961) histochemically demonstrated esterase activity in wax canals, so esterases at least could be carried mechanically above or under the waxy L2 from the wax canals.

The surface of a typical pheromone-sensitive sensillum trichodeum is indented with "pore funnels" about 100 nm in diameter that open into a pore canal. In *B. mori* there are two to eight pores per μm^2 on the pheromone-sensitive sensilla (Kaissling, K. 1971; Steinbrecht, R. 1973). There are two types of pheromone-sensitive sensilla in *T. ni.*

The pore canal below the pore funnel is about 8.5 nm diameter and 15–30 nm long. At its base it widens into a channel with a diameter of about 60 nm that opens to the dendritic chamber (Fig. 2). From the base of the pore canal extend pore tubules 10 nm in diameter, 500 nm long, that pass through an aqueous sensillum liquor toward the dendritic membrane of the olfactory cell. According to Steinbrecht, R. (1980) the pores, the pore canals, the pore kettles, and possibly the pore tubules are filled with the waxy L2 layer overlying the innermost L3 layer. However, because of its differential extractability (Hawke, S. 1970), the wax that fills the pore tubules in some sensilla may be different from the wax in other sensilla. This system of pores, pore canals, and tubules appears to be a lipoidal channel for the diffusion of pheromone molecules from the

epicuticular surface to the dendrite of the olfactory cell. There is some uncertainty whether the pore tubules are continuous or discontinuous through the sensillum liquor. New investigations comparing various methods of fixation suggest that at least some if not most of the tubules make contact with the dendritic surface (Steinbrecht, R. 1980; Keil, T. 1982).

Considering the structure and the composition of olfactory sensilla, the most likely transport path for the pheromone molecules is the following. First, the molecules adsorb to the antenna, diffusing along the surface of the epicuticle (Fig. 1). Some of the molecules that adsorb onto sensilla diffuse to a pore, to the pore tubules, and ultimately to the dendrite of a pheromone-sensitive olfactory cell. A fraction of these molecules are also degraded prior to reaching the cell. The diffusion coefficient of the bombykol on *Bombyx mori* cuticle has been calculated to be about 50 $\mu m^2\ s^{-1}$ (Steinbrecht, R. and Kasang, G. 1971). This means that a molecule reaches a pore canal within 2–10 ms on average after landing on the sensillum, and it reaches the dendrite within an average of 50 ms afterwards (Kaissling, K. 1971). The action potentials evoked by the pheromone molecules are initiated somewhat later. Kaissling, K. (1975) observed a mean reaction time of 200 ms to the first action potential, the elementary receptor potential preceding it by 50 ms.

4.2 Quantification of stimulus intensity at the olfactory dendrite

There have been two attempts to quantify the pheromone transport process (Kaissling, K. 1971; Mankin, R. and Mayer, M. 1983a,b). Both treat primarily the first step, deposition to the antennal surface, because the degradation and diffusion processes inside the sensillum are difficult to quantify. A complicating factor is that pheromone-sensitive sensilla usually have two or more olfactory cells which may respond dependently or independently to either the same or different pheromone components. It is thus difficult to specify which molecules reach which dendrites or to account for interactions among receptor cells. It can be assumed that no pheromonal degradation occurs during the stimulus period, or if a constant fraction of the adsorbed molecules reach the olfactory dendrites, then the total stimulus applied to all the pheromone-sensitive olfactory dendrites of the insect is approximately (Mankin, R. and Mayer, M. 1983a):

$$n_p/t = n_s N_a CSK, \qquad \text{(I)}$$

where n_p (number s^{-1}) is the mean rate of arrival of pheromone molecules at the dendritic membrane of the pheromone-sensitive cells, n_s is the number of pheromone-sensitive sensilla on both antennae, N_a is Avogadro's number, C (mole cm^{-3}) is the pheromone concentration in the stimulus air, S (cm^2) is the surface area of a pheromone-sensitive sensillum, and K is an empirically determined constant with a magnitude of about 1 cm s^{-1}. Typical values of n_sS and n_s are listed in Table 2. In *Trichoplusia ni*, for example, the value of n_sSK is about 2.8×10^{-2} $cm^3\ s^{-1}$. The male responds behaviorally to its sex pheromone at a concentration of about 8000 molecules cm^{-3} (Sower, L. *et al.*, 1971), so according to eqn I about 224 molecules per second are transported to the pheromone-sensitive olfactory cells.

It follows from eqn I that any quantitative estimates of pheromone deposition require detailed morphometric measurements of the antenna, and it is obvious from Table 2 that these details are not extensively known for any but a very few species (see also Chapman, R. 1982). One notable finding from the table is that the ratio of pheromone-sensitive sensillar area to flagellar area ranges narrowly from 0.13 to 3.75. The uniformity of this ratio is remarkable, despite the fact that the identification and estimation of the numbers of the pheromone-sensitive sensilla in some of the species were not precise. The surface-sculpturing of the flagellum, estimated by Steinbrecht, R. (1970) to increase the area three to four times, and the unknown surface area of the scales (in the Lepidoptera) could further narrow (or enlarge) this range of values. The amount of pheromone deposited onto sensilla of other Lepidoptera can be estimated from the flagellar area by assuming that the sensillar area approximates the flagellar area. Fortunately, such an approximation is sufficient for most purposes because the variation in total sensillar surface area is small relative to the large variations in pheromone concentration normally encountered in nature. Thus, for this purpose, additional detailed morphometric studies of antennae may not be necessary for other lepidopteran species.

Table 2: Surface area of the flagellum and olfactory sensilla of male insects

Species	Total flagellar area ($10^6\ \mu m^2$)	Sensillar type	Total sensilla/ insect (n_s)	Number of innervating cells per sensillum	Total sensillar area (n_sS) ($10^6\ \mu m^2$)	References
Insects having identified pheromones						
Cydia nigricana	1.97	s. trichodea*	24,360	2	7.39	Wall, C. (1978)
		s.basiconica (two types)	8,120	9 and 20	—	
Trichoplusia ni	7.2	s. trichodea				Mayer, M. *et. al.* (1981)
		I*	10,834	3	2.8	
		II	3,366	—	0.5	
		III	2,298	—	0.22	
Choristoneura fumiferana	1.08	s. trichodea				Albert, P. and Seabrook, W. (1973)
		I	—	8		
		II	—	—	—	
		III	—	—		
		IV*	368	7	1.39	
Lymantria dispar	66.92†	long s. trichodea*	44,070	—	55.38	Scheffler, H. (1975)
		short s. trichodea	1,536	—	0.82	
		s. basiconica	6,444	—	1.62	
Bombyx mori	45.09‡	long s. trichodea*	34,000	2	21.36	Schneider, D. and Kaissling, K. (1956, 1957); Steinbrecht, R. (1970)
		medium s. trichodea	5,000	2	1.26	
		s. basiconica	10,000	1–3	1.01	
Apis mellifera	7.29	s. placodea*	36,000	1–2	1.39	Esslen, J. and Kaissling, K. (1976)
		s. trichodea A	754	5–10	0.0426	
Manduca sexta	80.0	long trichoid setae	85,696	2	216.0	Sanes, J. and Hildebrand, J. (1976a,c)
		short setae	111,214	3	129.0	
Periplaneta americana	82.0	swB*	42,120	4	10.7	Schafer, R. and Sanchez, T. (1976); Schaller, D. (1978)
		swA	6,240	2–4	0.49	
		swC	4,680	2–4	1.54	
		dwA	6,240	2–4	0.49	
Insects not having identified pheromones						
Monochamus notatus	—	s. trichodea	730	7	0.361	Dyer, L. and Seabrook, W. (1975)
		s. basiconica	8,400	4	0.818	
Monochamus scutellatus	—	s. trichodea	800	8	0.396	Dyer, L. and Seabrook, W. (1975)
		s. basiconica	6,800	4	0.662	
Hylobius abietis	3.86	s. trichodea				Mustaparta, H. (1973)
		I	8,000–10,000	1–2	0.8	
		II	2,000	1	0.352	

Table 2: Continued

Species	Total flagellar area/*ns* (10^6 μm^2)	Sensillar type	Total sensilla/ insect (n_s)	Number of innervating cells per sensillum	Total sensillar area (n_sS) (10^6 μm^2)	References
Tenebrio molitor (female)	2.56	thick-walled peg organ	654	2	0.0208	Harbach, R. and Larsen, J. (1977)
		thin-walled peg organ	5,722	2	0.144	
		grooved peg organ	152	5	0.00223	
Antheraea polyphemus	—	long and short s. trichodea	110,000	1–3	158.96	Boeckh, J. *et al.* (1960)
		long and short s. basiconica	20,000	2–3	5.03	

* Sensilla having cells reported as pheromone-sensitive.
† Surface area of flagellum and branches measured for this publication.
‡ Includes surface area of branches.

4.3 Transduction of stimuli into generator potentials

Extreme difficulties are encountered in attempts to gather data for neurophysiological and biochemical studies of the olfactory neurons. The size and location of 0.3 μm diameter dendrites have so far eliminated any possibility of measuring their transmembrane (generator) potentials. Biochemical studies are difficult because the pheromone-receptive sensilla are usually interspersed with other chemosensilla having differing sensitivities. Also, the amounts of various macromolecules and membranes obtained are hardly sufficient for exacting studies. The dearth of definitive data sets few limits on the kinds of transductional mechanisms that theoretically are possible, so a large number have been propounded. For example, a chronological list and anecdotal treatment of 29 theories of transduction is given by Moncrieff, R. (1967).

The first treatment of olfactory transduction was apparently the account of Ogle, W. (1870), who thought that vibrations affected nasal epithelial pigment. Ramsay, W. (1882) was the second to propose a theory, this one based on heat (or infrared) vibrations. The theories of olfactory transduction applied to insects are listed in Table 3. Other major theories of olfactory transduction not directly linked to insects involve:

(1) macromolecular binding (Beets, M. 1970, 1974, 1975);
(2) physicochemical binding (Misra, T. *et al.*, 1968; Rosenberg, B. *et al.*, 1968);
(3) hole-puncturing (Davies, J. 1971);
(4) microtubule interaction (Atema, J. 1973, 1975);
(5) $Na^+ - K^+$ adenosine triphosphatase interaction (Koch, R. and Gilliland, T. 1977); and
(6) coacervates (Sperber, G. 1973, 1977).

It appears that most serious investigators now believe that transduction is initiated by a binding process between an odorant-ligand and a proteinaceous receptor molecule. We should, however, heed the caveat of Moncrieff that "...each theory has been put forward sincerely and represents the conclusions of the author after much work and meditation. Probably all contain some of the truth, but none all of the truth."

4.3.1 Hypotheses involving proteinaceous receptors

The most popular hypothesis of transduction of pheromones and other odorants at present is based on analogies with pharmacological and enzymatic theory, exemplified by the studies of bacterial chemotaxis (Koshland, D. 1980). It is thought that the pheromone interacts first with a protein "receptor" macromolecule (= acceptor of Kaissling, K. 1969) in, on, or comprising a portion of the receptor cell dendrite. There is no convincing evidence yet that such macromolecules exist, that they bind the odorant molecule, or that changes in the amount or rate of binding are in agreement with neurophysiological responses.

Several studies have implied or attempted directly to demonstrate macromolecular receptors in

Table 3: Theories of olfactory transduction applied to insects

Adduced transduction mechanism	Proponent(s)	Insect family
Macromolecular binding	Amoore, J. (1965, 1971)	Formicidae
	Klopping, H. and Meade, A. (1971)	
	Ryan, M. and Daly, P. (1978)	
	Norris, D. (1979)	Blattidae
	Kaissling, K. (1969, 1971, 1977a,b, 1978)	Bombycidae
	Kafka, W. (1974); Kafka, W. and Neuwirth, J. (1975)	Bombycidae
	Kikuchi, T. (1975)	Bombycidae
	Kikuchi, T. and Ogura, K. (1976)	Scolytidae
	Ohloff, G. and Giersch, W. (1980)	Drosophilidae
	Bestmann, H. *et al.* (1979)	Bombycidae
Steroid perimeter	Lee, G. (1979)	
Pharmacological, cyclic AMP	Villet, R. (1978)	
"Micro"-molecular binding	Norris, D. *et al.* (1970)	Blattidae
Sulfhydryl groups	Kosower, E. and Kosower, N. (1969)	Culicidae
Glutathione		
Infrared, radiative vibrations		
Vibrations	Fabre, J. (1912)	Saturnidae
	Grant, G. (1948)	Various Saturnidae
	Duane, J. and Tyler, J. (1950)	Saturnidae
Radiative	Laithwaite, E. (1960a,b)	Noctuidae
	Callahan, P. (1965, 1979)	Noctuidae
Absorption	Beck, L. and Miles, W. (1947)	Apidae
	Miles, W. and Beck, L. (1949)	Blattidae
Raman spectra	Wright, R. (1954, 1977)	Various families
Photochemical	Levengood, W. *et al.* (1973)	Noctuidae
Solvent-induced circular dichroism	Hayward, L. (1977, 1979)	Formicidae

antennae. Riddiford, L. (1970, 1971) reported experiments that she interpreted to demonstrate elution of receptor protein. Norris, D. (1979) outlined a number of investigations purportedly demonstrating a naphthoquinone receptor in various insect species. However, it is known that oxidizing agents such as naphthoquinone block impulse conduction in squid axons and affect the resting potential at low concentrations, causing spontaneous discharge at concentrations of about 0.2 mM (Brady, R. 1976). In other experiments, Villet, R. (1974) demonstrated that EAG responses of *Antheraea pernyi* pheromone were reduced by perfusion with reagents that block the amino ends of proteins at 2 mM concentrations and by perfusion with the sulfhydryl reagent *N*-ethylmaleimide at concentrations above 1 mM. In both instances the effects of perfusion were reversible. Reagents reacting with disulfide linkages were not effective at concentrations less than 10 mM and the effects produced were not reversible. The results of Villet, R. (1974) are of particular interest because the responses were tested with a known olfactory stimulus. Also of interest is the reversibility of the effects of the reagents; however, the large concentrations of reactants required suggest a non-specific effect. Access to the cells or active sites may be hindered, but this is not likely because pheromone-sensitive receptor cells have been stimulated by large, photoaffinity-labeled pheromones (Ganjian, I. *et al.*, 1978).

In studies on the effects of non-specific metabolic inhibitors on insect olfactory receptor cells, Levinson, H. *et al.* (1973) exposed the antenna of *Bombyx mori* to hydrogen cyanide (HCN) for 0.5–1.0 min. They reasoned that HCN probably induces a cytotoxic hypoxia through complexation with metalloenzymes. Such an exposure permanently eliminated both the standing and receptor potentials in pheromone-sensitive olfactory cells in *B. mori*, but had only limited, transitory effects on cells responding to geraniol in *Zygaena filipendulae* L., a moth of a group known to be highly tolerant to HCN and having large amounts of HCN sequestered in their tissues. The HCN may affect neurotransmitter activity, since in a potentially correlative experiment (Cardenas, H. and Zapata, P. 1980), NaCN affected the chemosensitivity of the carotid body.

The results of exposure of antennae to protein denaturants have been mixed. Formaldehyde produced a transitory effect in *Antheraea polyphemus* (Riddiford, L. 1970, 1971) and was stimulatory in *B. mori* (Kasang, G. 1971). Brief exposure to osmium tetroxide vapor, a reagent complexing with double bonds in lipids, preferentially reduced the receptor potential to bombykol compared to (*Z*)-10-tetradecen-l-ol (Kaissling, K. 1974). Further studies of the action of chemicals on olfactory receptors by Kaissling, K. (1980) revealed that (+)-*trans*-permethrin blocked impulse generation but not the receptor potential. The inhibition of olfactory cell responses by other chemicals is also discussed in detail by Kaissling, K. (1977a, 1980).

It has been reported that behavioral responses to odorants are inhibited by applying various sulfhydryl reagents, although the lack of specificity of these agents could be expected to produce a range of effects, including an indiscriminate reaction with receptor proteins as well as interference with other metabolic reactions necessary for routine cellular function (Galun, R. *et al.*, 1969; Koyama, N. and Kurihara, K.1971; Norris, D. *et al.*, 1971; Frazier, J. and Heitz, J. 1975). The EAG response also should be affected if indiscriminate metabolic reactions occur (Villet, R. 1974; Norris, D. and Chu, H. 1974; Frazier, J. and Heitz, J. 1975).

Indirect evidence for proteinaceous macromolecular receptors can be adduced from a number of other studies. Morphological studies indicate that there are a large number of intramembranous particles on the surface of the dendrites (Marshall, A. 1973; Steinbrecht, R. 1980) which are suggestive of clumps of receptor macromolecules, especially so in light of Steinbrecht's (1980) finding that the density of the intramembranous particles decreases toward the base of the ciliary segment (see also Menco, B. 1982). Kaissling, K. (1978) envisioned that the receptor molecule is located in the receptor cell membrane, presumably near the sites of close apposition to the pore tubules, each cell having only one type of receptor molecule, at least in *B. mori* (Kaissling, K.1976).

Further indirect evidence that the receptor moiety is a protein comes from findings that it is genetically encoded. Priesner, E. (1979) obtained data from EAG studies of Mendelian F_1 hybrids of two races of *Zeiraphera diniana* (Guenée), which indicate that the hybrids of the two races have equal sensitivity to the two different compounds eliciting quantitatively different EAGs by the parents. Although less conclusive than electrophysiological evidence, hybrid behavioral responses to pheromones further support this hypothesis. Both mating behavior and pheromone synthesis in interspecific and intraspecific hybrids in *Ostrinia nubilalis* (Hübner) and *Ips* spp., respectively, were mixtures of the behaviors of the parental strains (Lanier, G. 1970; Klun, J. and Maini, S. 1979). However, the relationship between behavior and pheromone perception is difficult to assess because the behavioral responses are affected by several factors in addition to perception (see section 6.2). The evidence that inheritance of pheromone production is controlled by an X-linked gene is interesting, especially if female hybrids must synthesize and males must simultaneously selectively discriminate and/or respond to the same blend (Grula, J. and Taylor, O. 1979). The inheritance of olfactory preference of a more general nature in *Dacus dorsalis* Hendel and *Drosophila melanogaster* Meigen does not appear to be controlled by genes on the X-chromosome (Fuyama, Y. 1978; Metcalf, R. *et al.*, 1979). Nonetheless, in the case of pheromones at least, synthesis, perception, and the behavioral response may have a relatively simple Mendelian basis.

There have been several attempts to correlate molecular parameters of the stimulus molecules with molecular parameters of a receptor moiety, particularly to explain single-cell discrimination processes. Presumably, the more points of attachment between a stimulus molecule and a receptor moiety, the greater the capability for discrimination. Kafka, W. (1976) has proposed that a nerve impulse arises from a multi-site odorant–receptor interaction, each with a low association energy of $0.6–1.0 \times 10^{-23}$ kcal per molecule. This hypothesis is similar to one proposed by Bestmann, H. *et al.* (1979), who conceptualized a more extensive atom-by-atom interaction of the molecule with the receptor. The energetic configuration of the "active site" relative to the functional groups of the pheromone is given by Kafka, W. (1974) and Kafka, W. and Neuwirth, J. (1975). A bi-functional receptor site was envisioned by Kikuchi, T. (1975) based on the statistical relationship between the EAG response and the probability that various pheromone

analogs occur in a particular conformation. Similar comparisons by Kikuchi, T. and Ogura, K. (1976) that were based on behavioral responses appear to be less satisfying. A bi-functional attachment site has been proposed for the alarm pheromone, undecane, in *Lasius fuliginosus* Latreille (Dumpert, K. 1972) and a four-point attachment site was proposed for (*Z*)-11-tetradecen-l-ol acetate in *Argyrotaenia velutinana* (Roelofs, W. and Comeau, A. 1971a).

Some investigators hypothesize that, in addition to the stimulus amplification occurring at transduction, another mechanism must exist where small numbers of molecules such as hormones, carotene, or adenosine 3′,5′-cyclicmonophosphate (cyclic AMP) further amplify the response of the receptor cell to odorant molecules. Rothschild, M. (1978) suggested that carotene amplifies transduction or perception by peripheral or central nervous system (CNS) action. She instigated an experiment performed by K.-E. Kaissling on normal and carotene-deficient *Hyalophora cecropia*. However, he found that individual pheromone-sensitive sensilla of wild and carotene-deficient males were not different in neurophysiological sensitivity to native pheromone. Other experiments cited by Rothschild also failed to confirm a relationship between carotene and sensory functions with the exception of vision.

Hormones are linked only speculatively to receptor cell physiology and stimulus amplification mechanisms (Truman, J. and Riddiford, L. 1974, 1977) (see section 3.1). Although the mode of action is imperfectly known, Küppers, J. and Thurm, U. (1975) suggest the ion transport mechanism, generating the transepithelial potential, to be under hormonal control. Their reasoning is based on the finding that 5-hydroxytryptamine, which mimics hormone action in organs regulating salt and water balance, effected a 20–25% difference in the transepithelial potential. They believe this difference reflects the activation of the transport mechanism itself and not the permeability change *per se*.

Presently it is difficult to make a case for cyclic AMP in chemosensory physiology because its many functions have not been clearly identified. A large body of evidence implicates cyclic AMP in a role as an amplification mechanism in receptor cells (Robison, G. *et al.*, 1971; and many others). To date, the only investigation of an effect of cyclic AMP on the olfactory response to pheromone was by Villet, R. (1978), who found the EAG response of *Antheraea pernyi* to vary in sensitivity after perfusion with various chemicals functionally related to cyclic AMP. The greatest enhancement of the response occurred by perfusing the antennae in cyclic AMP or by inhibiting phosphodiesterase activity with sodium citrate. Cyclic AMP activity has been demonstrated in dendritic terminals and in supporting cells of contact chemoreceptors of *Phormia regina* (Meigen) (Felt, B. and Vande Berg, J. 1977). Phosphodiesterase activity was localized along axonal microtubules (Vande Berg, J. 1975). Cyclic nucleotides and phosphodiesterase inhibitors affected the response to sucrose (Daley, D. and Vande Berg, J. 1976).

The findings by Villet and Vande Berg and associates suggest that microtubules in the receptor cell dendrites may have more than structural significance, and a theory of olfactory transduction has been proposed based on microtubule interactions (Atema, J. 1973, 1975). Microtubules are continuous with the basal ciliary apparatus in the receptor cell dendrites (Slifer, E. and Sekhon, S. 1969; Steinbrecht, R. 1980), and microtubule disrupters, vinblastin and colchicine, influence both the resting potential (Witte, H. 1980) and the responsiveness of contact chemoreceptors of *Phaenicia sericata* (Meigen) to sucrose (Matsumoto, D. and Farley, R. 1980). However, unless cyclic AMP, its metabolic relatives, or phosphodiesterase can be demonstrated to be localized on or within the dendritic membrane of the olfactory receptor cell, it is difficult to ascribe a role in the transductional process to it. Likewise, a functional relationship of microtubules and microfilaments to the transductional process is difficult to conceptualize and it seems that unless the microfilaments contact or closely approach the dendritic membrane their function may be only to maintain the structural integrity of the cell, as proposed by Loor, F. (1976).

Thus, there is a consensus that the pheromone molecule, reaching the vicinity of the receptor site by diffusion, interacts weakly with a receptor macromolecule at two or more positions. The interaction may directly affect the ion transport across the dendritic membrane and may have other effects on microtubules. However, such a receptor molecule has never been demonstrated unambiguously *in vivo*

or *in vitro* from a purely olfactory system in any organism save micro-organisms. Consequently, the transduction hypotheses based on other mechanisms cannot be completely ruled out at this time.

4.3.2 Hypotheses based on infrared resonance processes

All of the theories based on direct transduction of infrared vibrations emanating from pheromone molecules have fallen into disfavor because of their fundamental difficulty in explaining the differential perception of optical isomers which have identical infrared spectra (Russell, G. and Hills, J. 1971). It has been unambiguously demonstrated that optical isomers have different odors to humans (Friedman, L. and Miller, J. 1971) and to insects (Silverstein, R. 1979) (also see section 5.2).

To illustrate the intensity of the debate between infrared-based and proteinaceous receptor-based transductional hypotheses, it is instructive to contemplate carefully a series of reports purportedly "proving" and/or "refuting" these two competing and mutually exclusive theories. Using ant alarm-pheromone behavior, Amoore, J. *et al.* (1969) demonstrated a highly significant correlation between alarm activity and molecular shape, and challenged R. H. Wright, having a competing infrared-based theory, to a test using the same chemicals and behavioral data (Amoore, J. 1971). In a rebuttal a year later (Wright, R. 1972; Wright, R. and Brand, J. 1972), evidence was presented that certain infrared frequencies were correlated with the observed behavior. A more complete assessment of structure–infrared-behavior studies by Blum, M. *et al.* (1971), however, found no agreement between the criterion behavior and the IR spectrum of the molecule. They did not assess size and shape effects. Finally, using the same behavioral data as Amoore, J. *et al.* (1969), Hayward, L. (1977, 1979) attempted to answer the difficulty of optical isomers for adherents of radiative transduction.

The debate above is instructive more generally than just in the context of the two theories because it points out the pitfalls inherent in over-interpreting data. The behavioral responses were fact, but the ant alarm-behavior was too far removed from the receptor cell event by intervening neural processing for it to be of conclusive use to decipher transduction, as is noted in section 5.3. As a result, both proponents of the competing theories could consider the same data to have "proven" each of two mutually exclusive hypotheses, while in reality the data were inconclusive for either hypothesis.

4.3.3 Quantification of the generator potential

From a theoretical viewpoint the most appealing of the proposed transductional hypotheses are those that incorporate the concept of a proteinaceous receptor because the physical mechanisms involved are fundamental in a number of biological processes. A quantitative model for the physiological and electrical interactions in the olfactory cell can be derived by applying the law of mass action to the presumed odorant–receptor interaction (Kaissling, K. 1969, 1971, 1972, 1974, 1976). In the model it is assumed that a stimulus molecule, S, combines with a receptor site, R, to form a complex, RS, which opens ion channels, depolarizing the receptor cell membrane. This depolarization is the generator potential that directly drives the production of action potentials. Some odorants hyperpolarize the cell membrane, but no pheromones to date have shown hyperpolarization effects. The forming of the receptor complex is not well understood, but from studies of pheromone receptor cells in *Bombyx mori* (Kaissling, K. 1974), it appears that different parts of the pheromone molecule interact with corresponding sub-sites on the receptor protein and that each sub-site serves a different function. Kaissling found that a weak pheromonal stimulus produces a fluctuating receptor potential (a relative measure of the generator potential) resulting in an irregular spike frequency, such as would be expected if a few molecules each activated a large contribution to the generator potential. By contrast, a stronger stimulus of a pheromonal analog can produce spikes of the same average frequency as the pheromone, but at more regular intervals, with the generator potential having fewer irregularities. This would be expected if a large number of molecules each activated a small contribution to the generator potential. In other cases the response to a high dose of a pheromonal analog can be much the same as the response to a low dose of pheromone, such as would be expected if the molecules have a low affinity for the binding site.

To account for the fact that different contributions to the generator potential occur when different odorant molecules form a receptor complex, it can be assumed that the receptor complex, RS, is converted to an activated form, R*S, which triggers an odorant-dependent increase, g_i, in the conductance across the receptor cell membrane. The extent of activation depends upon the interactions of the odorant molecules with the different subsites in the receptor complex. Presumably, each activated complex controls a single ion channel with a maximum conductance increase of about 10^{-10} mhos (Kaissling, K. 1976). The total increase in the conductance from all of the activated complexes

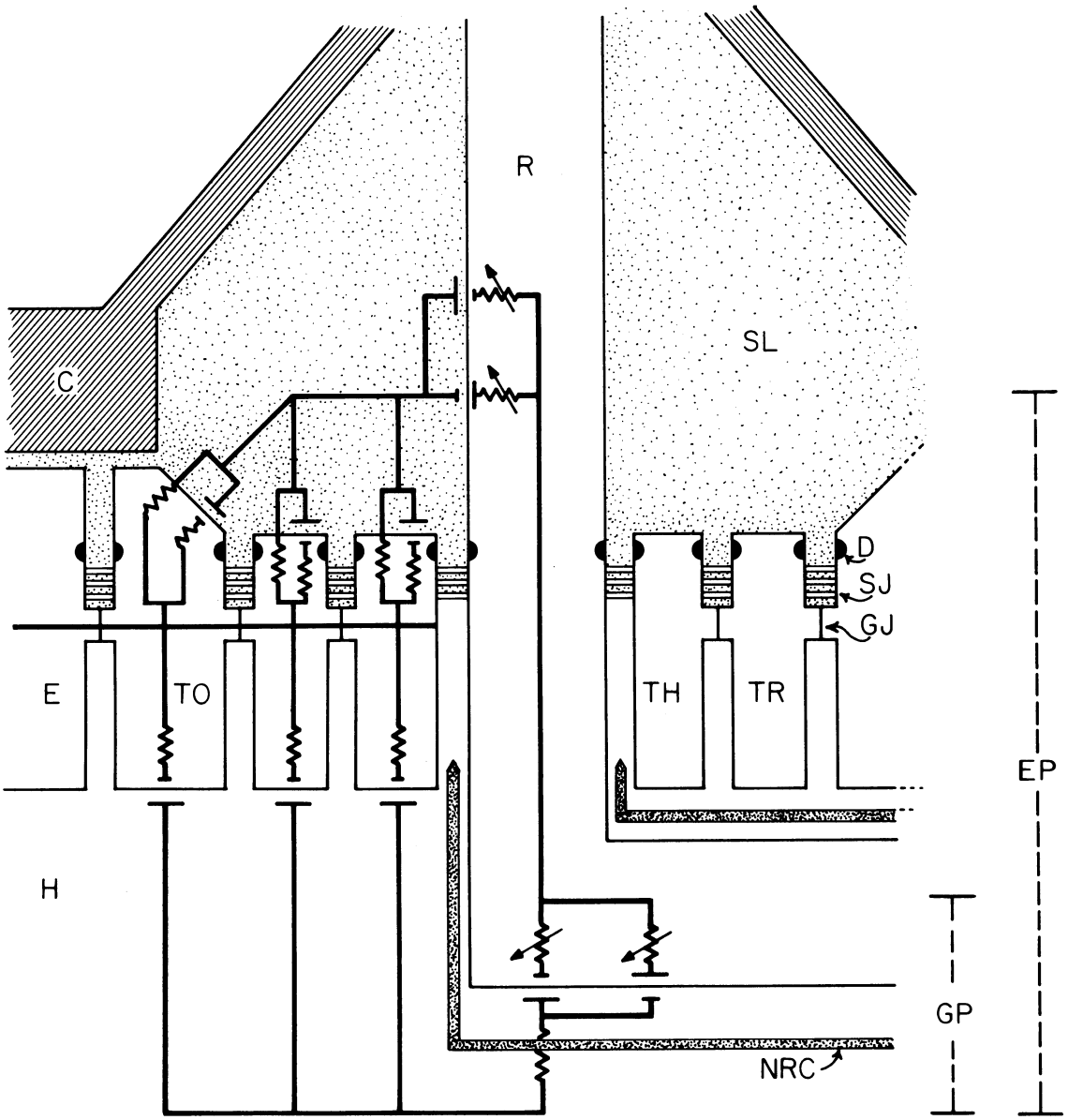

FIG. 3. Diagram of the electrical characteristics of the pheromone receptor cell and adjacent epithelial cells. Nomenclature: C = cuticle; D = desmosome; E = epithelial cell; EP = transepithelial potential; GJ = gap junction; GP = generator potential; H = hemolymph; NRC = neurilemma cell; R = receptor cell; SJ = septate junction; SL = sensillum liquor; TH = thecogen cell; TO = tormogen cell; TR = trichogen cell.

results in a change, ΔV, in the membrane potential. Apparently, the potential difference induced by a single bombykol molecule activating a receptor complex is sufficient to cause the generation of an action potential in *B. mori* (Kaissling, K. and Priesner, E. 1970; Kaissling, K. 1974, 1980). Other stimulatory molecules would be expected to be less efficient in activating the receptor complex, inducing a smaller conductance increase, and producing fewer action potentials.

No direct measurements have been made of the generator potential or the relationship between the generator potential and the total conductance. The current concept of this relationship is based on inferences from the morphology of pheromone receptor cells and the lumped electrical properties of mechano- and pheromone-receptor cells and their nearest neighbors, the outer (tormogen), intermediate (trichogen), and inner (thecogen) sheath cells (Fig. 3) (Kaissling, K. 1971; Thurm, U. and Küppers, J. 1980; Kaissling, K. and Thorson, J. 1980). All four types are modified epithelial cells, apparently derived from a single parent (Sanes, J. and Hildebrand, J. 1976a). They are tightly joined at their apical membranes just below the cuticle by high-resistance septate junctions and desmosomes. Consequently, the electrical resistance across the whole epithelium is large. The three non-neural cells of each sensillum are electrically connected with low-resistance gap junctions. The resistance between any two epithelial cells, except for the olfactory receptor cell, is small. The transepithelial specific resistance, i.e. the resistance between the inside and outside faces of a $1\,cm^2$ area of epithelium (Geddes, L. 1972), is 3000 Ω-cm^2 (Thurm, U. 1974). There are no gap junctions between the olfactory cell and the three surrounding cells, so the electrical diagram in Fig. 3 shows two parallel pathways for current — one path through the olfactory cell and one transepithelial pathway.

The trichogen and tormogen cells have a highly folded cell membrane on the side bounded by the sensillum liquor space (Zacharuk, R. 1980). These membranes may contain an electrogenic pump (Rick, R. *et al.*, 1976; Thurm, U. and Küppers, J. 1980) that transports K^+ ions to the sensillum liquor and contributes to the +30 mV potential between the sensillum liquor and hemolymph (Kaissling, K. and Thorson, J. 1980).

For the electrical circuit in Fig. 3, the equation relating the generator potential to the conductance change is

$$\Delta V = \Delta V_m/(1 + g_0/\Delta g), \qquad \text{(II)}$$

where ΔV_m is the maximum potential difference, which occurs as Δg approaches infinity, and g_0 is the membrane conductance in the unstimulated state. It should be noted that eqn II does not take into account the cable properties of the dendrite. The spread of current through the receptor cell is strongly affected by the cell diameter. In *Bombyx mori* the diameter of the distal portion of the dendrite is 0.2–0.4 μm (Steinbrecht, R. 1980). Between the distal and proximal portions of the dendrite, the proximal having a diameter of 0.8 μm, there is a "ciliary" region with a diameter of 0.3 μm. Kaissling, K. (1971) derived an equation in which the cell is divided into two sections, one representing the outer dendrite and the other the inner dendrite, soma, and axon. The resultant potential–conductance relationship is similar to eqn II except that it is somewhat flatter, depending on the length of the outer dendrite.

A model of the processes occurring in the receptor cell can be derived from the studies above, but the application of such a model is problematic because many of the model parameters have not been measured to date. In deriving the model, first, the interaction between the stimulus molecules and the receptor molecules is calculated according to the reaction sequence:

$$[R] + [S] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [RS] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} [R^*S], \qquad \text{(III)}$$

where the brackets denote concentration in units of number of molecules/receptor cell, k_1 and k_{-1} are the forward and backward velocity constants for the first reaction, with units of molecules^{-1}s^{-1} and s^{-1}, respectively, and k_2 and k_{-2} are the forward and backward rate constants for the second reaction, with units of s^{-1}. The steady-state conditions are given by

$$d[R]/dt = k_{-1}[RS] - k_1[R][S] \qquad \text{(IV)}$$

and

$$d[RS]/dt = k_1[R][S] + k_{-2}[R^*S] - (k_{-1} + k_2)[RS]. \qquad \text{(V)}$$

Next, we note that the total conductance across the cell membrane is the product of the conductance per ion channel times the concentration of activated complex,

$$\Delta g = g_i[R^*S]. \quad \text{(VI)}$$

Thus, to relate eqn II to the stimulus concentration, [S], requires that we determine [R*S] from eqns IV and V as a function of [S]. By defining

$$[R_{tot}] = [R] + [RS] + [R^*S], \quad \text{(VII)}$$
$$K_1 = k_{-1}/k_1,\ K_2 = k_{-2}/k_2, \quad \text{(VIII)}$$

and

$$Q = g_0/(g_i[R_{tot}]), \quad \text{(IX)}$$

where Q is essentially the ratio of the unstimulated conductance to the maximum conductance of the cell, we obtain the final result:

$$\Delta V = \Delta V_m/(1 + Q + K_2Q + K_1K_2Q/[S]). \quad \text{(X)}$$

The relationships expressed by eqn X are in agreement with what might be expected intuitively. Increasing K_1, i.e., either by increasing the rate of dissociation of RS complexes or by decreasing the rate of formation, shifts the entire hyperbolic curve of potential *vs.* concentration to the right, toward increasing concentration. Increasing K_2, i.e., either increasing the rate of dissociation of R*S complexes or decreasing the rate of formation, flattens the curve and shifts it to the right. Decreasing the conductance of an ion channel, g_i, has a similar effect as increasing K_2, but the effect is more pronounced, depending on the magnitude of Q relative to K_2. The most significant result is that the three parameters, k_1, k_2, and g_i, affect the generator potential in different ways; thus, in modeling the specificity of pheromone receptor cells to different compounds, it is necessary to specify a binding constant, an activation constant, and an average increase in conductance per activated ion channel.

Recently, several models for bacterial chemoreceptors have been proposed that may be of interest in pheromone receptor cell studies as well. For example, Koshland, D. (1980) proposed that the stimulus could act simultaneously on a different receptor protein to produce an inhibitor of the formation of active complex, R*S. In this case the dissociation "constants" become functions of the inhibitor concentration, [*I*], of the form

$$K = A(1 + [I]/B), \quad \text{(XI)}$$

where A and B are constants. In certain systems of this type, the resultant stimulus–response curve is a power function, such as is often obtained in psychophysical studies (Stevens, S. 1975). Since the measured "receptor" potential (and thus presumably the generator potential) and the spike frequency in *Bombyx mori* are power functions of the pheromone intensity over a wide range of concentrations (Kaissling, K. 1971), such a modification to eqn X should perhaps be investigated in some detail.

4.4 Coupling of the generator potential to action potentials

The polarity of the spikes induced in a receptor cell indicates that the spike generator lies underneath an extracellular diffusion barrier that also has a high resistance electrically (Thurm, U. and Küppers, J. 1980). Because of this resistive zone, there is no extracellular shunt, only a path through the sheath cells (more resistive than through the activated receptor cell). The change in the transepithelial potential upon stimulation is thus equivalent to the generator potential (the "receptor potential" of Kaissling, K. and Thorson, J. 1980). According to Thurm, U. and Küppers, J. (1980), the spike frequency is roughly proportional to the change in the transepithelial potential. However, in *B. mori* the spike generator appears to respond primarily to rapid negative changes in the transepithelial potential and less to a constant depolarization (Kaissling, K. and Thorson, J. 1980). As the pheromone concentration rises the spike output becomes progressively more phasic–tonic in character. Because this phasic–tonic output occurs at concentrations considerably above the pheromonal threshold of behavior, we will not further consider its significance and discuss only the case where the spike frequency is proportional to the change in the transepithelial potential.

To express the mean spike frequency in terms of the pheromone concentration, it is convenient to first simplify eqn X by defining a dissociation constant:

$$K_b = K_1/(1 + (1/K_2) + (1/K_2Q)), \quad \text{(XII)}$$

and

$$\Delta V'_m = \Delta V_m/(K_2Q + Q + 1). \quad \text{(XIII)}$$

Substituting eqns XII and XIII into eqn X yields

$$\Delta V = \Delta V'_m/(1 + K_b/[S]). \quad \text{(XIV)}$$

The proportionality between the generator potential and spike frequency is approximately

$$F = F_m \Delta V/\Delta V'_m, \quad \text{(XV)}$$

where F is the mean spike frequency for a generator potential difference of ΔV, and F_m is the mean maximum frequency, occurring when $\Delta V \rightarrow \Delta V'_m$. Accordingly, the regression of concentration on spike frequency is

$$F = F_m/(1 + K_b/[S]). \quad \text{(XVI)}$$

Equation XVI is somewhat inadequate for describing the curves for spike frequency *vs.* pheromone concentration that have been determined experimentally. Generally, the experimental curve is a power function which is flatter than the theoretical hyperbolic curve, as is also the case for the generator potential (Mankin, R. and Mayer, M. 1983b). Another equation that represents the frequency–concentration curve is a power function of the form:

$$F = b_1([S] + b_2)^b, \quad \text{(XVII)}$$

where b_1, b_2 and b are regression constants determined by a non-linear regression analysis. This relationship is used frequently in vertebrate psychophysics because the exponent, b, is independent of the stimulus and response units and thus is reflective of the inherent characteristics of the olfactory cell (Mankin, R. and Mayer, M. 1983a).

4.5 Transmission of action potentials to the antennal lobes of the deutocerebrum

Once an olfactory stimulus is transduced into a series of action potentials by an olfactory receptor cell, the information encoded therein proceeds to the higher centers of the brain via the antennal lobes of the deutocerebrum (Jawlowski, H. 1936; Steinbrecht, R. 1969). Axons from the pheromone-sensitive cells, other types of olfactory cells, and the mechano- and contact chemoreceptors on a single antenna collect into a set of bundles called the antennal nerve. Here we discuss briefly the morphology and the neurotransmitters of the antennal nerve before treating the central processing of olfactory information by interneurons in higher brain centers.

Most of the axons in the antennal nerves project into one or more of a small number of clumps of synaptic connections called glomeruli in the ipsilateral antennal lobes of the deutocerebrum (Ernst, K. *et al.*, 1977; Hildebrand, J. *et al.*, 1980). Additional axons bypass the deutocerebral glomeruli and project into the tritocerebrum. These probably derive from antennal contact chemoreceptors because the tritocerebrum is concerned mainly with feeding (Horridge, G. 1965). There has been some uncertainty in the past whether the axons in the antennal nerve fuse before reaching the deutocerebrum, but it is now generally accepted that no fusion occurs. Wigglesworth, V. (1959) believed from light microscopic studies that a 15 : 1 fusion of axons from olfactory receptor cells of *Rhodnius prolixus* Stål had occurred in the antennal nerve when it had passed the base of the terminal segment. Other investigators also proposed that fusion or anastomosing of peripheral sensory cells occurred (Dethier, V. *et al.*, 1963; Bullock, T. and Horridge, G. 1965). Because of the obvious implications of axonal fusion on the transmission of qualitative and quantitative information to the brain, Steinbrecht, R. (1969) investigated this question in *Bombyx mori* as well as in *R. prolixus*. Individual larger-diameter axons were found to have glial sheaths, and the small fibers were gathered into bundles ensheathed by glial cells (Steinbrecht, R. 1969; Schafer, R. 1973). Steinbrecht concluded, without overwhelming evidence, that cross-excitation in these naked axons was negligible. Consequently, he proposed that no anastomosing of individual cells occurred in the antennal nerve and that the spatial requirements for the large numbers of axons were alleviated by their small diameter (0.3 μm).

The principal neurotransmitter in axons of the antennal nerve of *Leucophaea maderae* (F.) and *Manduca sexta* is acetylcholine. Dopamine is present at only 10% of the level of acetylcholine. Traces of other neurotransmitters are present (Schafer, R. 1973; Sanes, J. and Hildebrand, J. 1976b). It is logical that acetylcholine is the neurotransmitter of the pheromone receptor cells because they outnumber the other types of receptor cells on the antenna. Other neurotransmitters identified in the brain tissue of *M. sexta* are γ-aminobutyric acid

(GABA), 5-hydroxytryptamine, histamine, dopamine, norepinephrine, tyramine and octopamine. The antennal lobes produce and store, besides acetylcholine, GABA, histamine, and tyramine (Maxwell, G. *et al.*, 1978). It would seem that the GABA observed, apparently near the glomeruli (Frontali, N. and Pierantoni, R. 1973), is not a neurotransmitter of the peripheral olfactory cells because the antenna cannot synthesize detectable amounts of GABA (Sanes, J. and Hildebrand, J. 1976b). Instead, GABA may be utilized in unidentified interneurons (see, e.g., Kerkut, G. *et al.*, 1969).

4.6 Processing of olfactory information by interneurons in the deutocerebrum and higher brain centers

The organization of the major areas of the brain that process olfactory input is schematized in Fig. 4. The general organization, anatomy, and microanatomical details of the brain are described in detail by Strausfeld, N. (1976). Microanatomical details of synapses in the deutocerebrum of *Locusta migratoria* (L.) were investigated by Schürmann, F. and Wechsler, W. (1969, 1970), Ernst, K. *et al.* (1977) and Boeckh, J. *et al.* (1970). Among the noctuid moths, only the brain of *Prodenia litura* (F.) has been investigated in any detail (Srivastava, B. 1969), although other lepidopteran brains have been investigated (Newport, G. 1832, 1836; Bretschneider, F. 1924; Nordlander, R. and Evans, J. 1969; Pearson, L. 1971).

The glomeruli that terminate the antennal nerve appear to be organized in an invariant manner among individuals of *Blaberus craniifer* Burm. (Chambille, I. *et al.*, 1980; Rospars, J. and Chambille, I. 1981). Some glomeruli may be specialized for processing the output from specific types of olfactory receptor cell. One such specialized glomerulus is a large macroglomerulus that is found in males of a number of species near the entrance of the antennal nerve to the deutocerebrum. The macroglomerulus has been observed in *Periplaneta americana* (L.), *Antheraea pernyi*, *Antheraea polyphemus*, *Manduca sexta*, and *Blaberus craniifer* (Jawlowski, H. 1954; Schaller, D. 1977, unpublished citation by Boeckh, J. and Boeckh, V. 1979; Hildebrand, J. *et al.*, 1980; Chambille, I. *et al.*, 1980; Matsumoto, S. and Hildebrand, J. 1981). In electrophysiological studies, Boeckh, J. and Boeckh, V. (1979) reported that no responses to pheromonal stimuli by male *P. americana* were recorded from any region of the deutocerebrum except the macroglomerulus. Earlier, Yamada, M. (1971) and Waldow, U. (1977) had reported similar responses to male and female-derived odors in the same insect. It is probable that Yamada, M. (1971) recorded from the macroglomerulus because a number of fibers highly specific for crude aggregation pheromone were observed; interpretation was difficult at the time because no structure recognizable as a macroglomerulus was identified at that time. Waldow's recordings, although not specifically localized, were from "... the frontolateral deutocerebrum between the protocerebrum and the point of entry of the antennal nerves". It thus appears that the macroglomerulus is the major terminus of pheromone-sensitive receptor cells.

The glomeruli in the deutocerebral lobes of *P. americana* comprise synapses from at least three functional types of neuron:

(1) the ipsilateral peripheral receptor cells;
(2) interneurons that connect to other glomeruli in the ipsilateral deutocerebral lobe; and
(3) interneurons that project to the protocerebral lobes and to the calyces of the mushroom bodies through the *tractus olfactorio globularis* (Strausfeld, N. 1976; Ernst, K. *et al.*, 1977; Hildebrand, J. *et al.*, 1980; Burrows, M. *et al.*, 1982).

The mushroom bodies are complex masses of neuropile having two lobes designated α and β. Contralateral connections between the mushroom bodies have been demonstrated histologically (Strausfeld, N. 1976; Masson, C. 1977). Obtaining neurophysiological recordings from the mushroom bodies has proven to be difficult; however, recordings of olfactory responses in the protocerebral lobes have been obtained from *Boettcherisca peregrina* (Robineau-Desvoidy) by Mimura, K. *et al.* (1969) and from *Apis mellifera* (L.) by Suzuki, H. and Tateda, H. (1974), Suzuki, H. (1975b) and Suzuki, H. *et al.* (1976).

The anatomical studies of Pearson, L. (1971) on *Sphinx ligustri* (L.) and Vowles, D. (1955) on bees and ants are suggestive of an olfactory route from the β lobe of the mushroom bodies to the

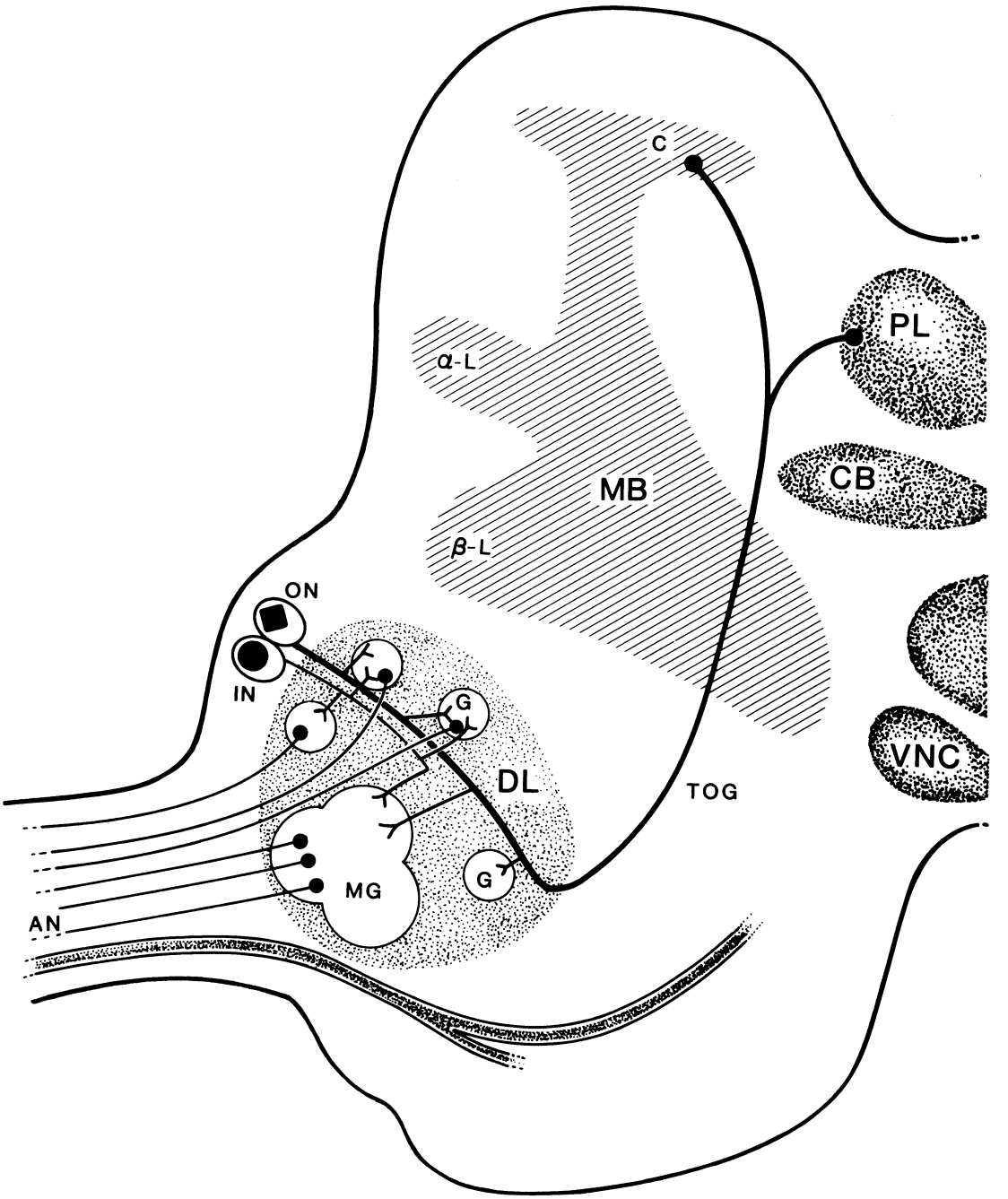

FIG. 4. Schematic sagittal diagram of the antennal lobe and deutocerebral tract in male moths and cockroaches. Nomenclature: AN = antennal nerve; C = calyx of a mushroom body; CB = central body; DL = deutocerebral lobe; G = glomerulus; IN = interneuron; MB = mushroom bodies with α and β lobes; MG = macroglomerulus; ON = output neuron; PL = protocerebral lobe; TOG = tractus olfactorio globularis; VNC = ventral nerve cord.

suboesophageal ganglion. No neurophysiological demonstration of this linkage has been reported, but should such a route exist, it obviously would be of great interest as a link between odor discrimination and behavior. Maynard, D. (1956) found indirect support of such a link in *Periplaneta*

americana. Its existence in other insects remains to be demonstrated.

The time necessary for an insect to respond behaviorally to an olfactory stimulus, the reaction time, is an indirect measure of the amount of central processing occurring during perception. The reaction time to sex pheromone was measured in *Bombyx mori* by Kaissling, K. and Priesner, E. (1970), who found that the most rapid reaction time was 100 ms, with an average of 400 ms. The minimum reaction time compares to the very rapid evasive behavior within 75–252 ms (average = 140 ms) by moths to pulses of sonar from bats, and cockroach evasive behavior of 54 ms (Roeder, K. 1963).

The reaction time to pheromone may also be compared to the 177 ms required to elicit simple movement of the ipsilateral antenna of *Apis mellifera* to odor stimulation (Suzuki, H. 1975a). Movement of the contralateral antenna required 248 ms. This short reaction time to pheromone suggests that a relatively small number of synapses are involved in the initial processing of pheromonal stimuli, as well as a small number of efferent synapses, and is supportive of the quantitative phenomenological model of pheromone response by Mankin, R. and Mayer, M. (1983a,b) (see also section 6.2.1). Certainly, in *A. mellifera* not all of the antennal afferent fibers terminate in the antennal lobe of the deutocerebrum, and small glomeruli exist in the dorsal lobe of the deutocerebrum as well as in the calyx of the corpora pedunculata (mushroom bodies) and tritocerebrum (Pareto, A. 1972). We might suspect then that in some insects a very simple activation pathway may exist from the macroglomerulus to the efferent fibers in the ventral nerve cord (Fig. 4). Such speculation is supported somewhat by U. Waldow's (1977) finding of >50 ms latencies for pheromone-sensitive type Ia neurons near the macroglomerulus. This activation pathway might bypass to some extent much of the central processing in the mushroom bodies and elicit a twitch or alerting movement by the antenna as described by Schwinck, I. (1955, 1956) for *Bombyx mori* and for *Trichoplusia ni* by Ignoffo, C. *et al.* (1963) and Shorey, H. (1964). With noctuid moths this arousal behavior is rapid and proceeds often-times to a "shudder" or wing-fanning. Simultaneous with this rapid neural routing would be a less rapid second route to higher centers in the CNS. It is the second route by which the insect discriminates between a "favorable" or "non-favorable" stimulus (see section 6.2.1).

4.7 Quantification of pheromonal stimulus in the deutocerebrum and efferent fibers

Except at the glomerular level there is little direct evidence from insects concerning the quantification of the pheromonal information passing to the deutocerebrum and later stages of processing. The anatomy of the macroglomerulus suggests that the mechanism by which some insects attain their extreme sensitivity to pheromone may be the convergence of a large number of pheromonal receptor cells into a small number of pheromone-sensitive interneurons. Indeed, Boeckh, J. and Boeckh, V. (1979) found essentially an increase of two orders of magnitude in their ability to measure pheromone responses in deutocerebral neurons of *Periplaneta americana*. There was a significant response to 10^{-4} or 10^{-5} μg loads at the periphery and 10^{-6}–10^{-7} μg loads in the deutocerebrum. Presumably this occurs because at very low levels of pheromone there is a finite and low probability of activating the single peripheral receptor cell under electrophysiological scrutiny (Kaissling, K. and Priesner, E. 1970), and the recordings from a convergence of 100 cells theoretically would be 10 times more sensitive than from an individual peripheral receptor cell (Mankin, R. and Mayer, M. 1983a).

After the summing process at the first synapse of the macroglomerulus, the pheromonal response undergoes considerable additional processing in the CNS. An analogy with olfactory perception in humans suggests indirectly that the information-processing steps are relatively linear (Stevens, S. 1975). In humans unadapted to a stimulus both perception and evoked neurophysiological activity are power functions of stimulus intensity, which has been interpreted to be evidence of linear processing. As in humans, the response of insect pheromone receptor cells may be represented by a power function of the pheromone concentration (see section 4.4). One cannot ask an insect what it perceives except by an imperfect bioassay, and pheromone bioassays are usually analyzed in terms of probit curves rather than power functions. Nevertheless,

an inspection of published pheromone bioassays of insects reveals that such bioassays can indeed be represented by power functions, as indicated in the next paragraph. To the extent that perception is measured by a bioassay, it can thus be inferred that the perception of pheromone is a power function of the stimulus concentration, and also that the CNS is linearly processing the response provided by the olfactory cells.

An example of a power function response in a bioassay can be obtained from a study by Mankin, R. *et al.* (1980b) on *Plodia interpunctella* (Hübner). In Fig. 5 the percentage response of males to different concentrations of a major component of this insect's sex pheromone is presented for bioassays at two different temperatures.

Both curves were originally described via probit analysis, but they also can be described by power functions. At 34° the mean percentage response is given by the equation

$$R = 70.25(D - 3.12 \times 10^{-4})^{0.142}, \quad \text{(XVIII)}$$

where R is the percentage response and D is the dose of pheromone applied to the dispenser. The coefficient of determination is $r^2 = 0.99$. At 23° the mean percentage response is

$$R = 24.73(D - 1 \times 10^{-4})^{0.222}, \quad \text{(XIX)}$$

also with $r^2 = 0.99$. It should be noted that the two exponents, 0.142 and 0.222, are not statistically different at the 95% confidence level. This is to be expected since, according to Stevens, S. (1975), the exponent is probably a measure of transductional processes. The transductional steps would not necessarily be altered by changing the temperature from 23 to 34°.

We conclude that, at least for pheromones, quantitative processing of inputs to the antennal lobe occurs in much the same way as presented in Fig. 6. The inputs from a large number of pheromone-receptive neurons converge at a single locus, such as a macroglomerulus, where they are summed. This step is represented by the central processor box in the figure. Subsequent central processing required for qualitative discrimination of pheromone blends is discussed in the following section (section 5). It appears that little additional processing is required to initiate the simplest orthokinetic reactions,

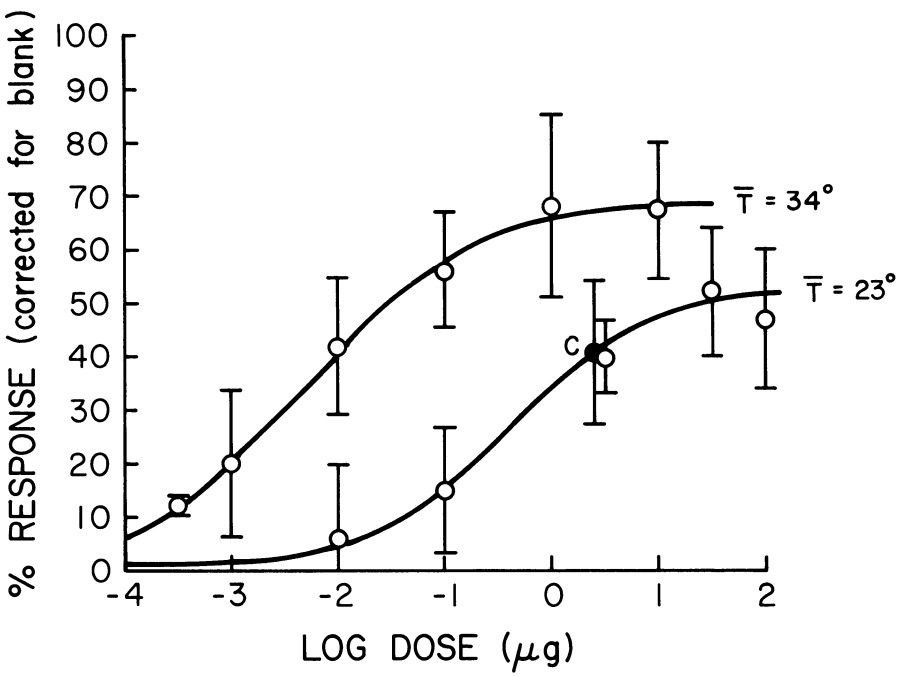

FIG. 5. Percentage of male *Plodia interpunctella* flying upwind at two different temperatures in response to stimulation by the major component of its sex pheromone. The bars denote the 95% confidence interval. The lines were fitted by probit analysis.

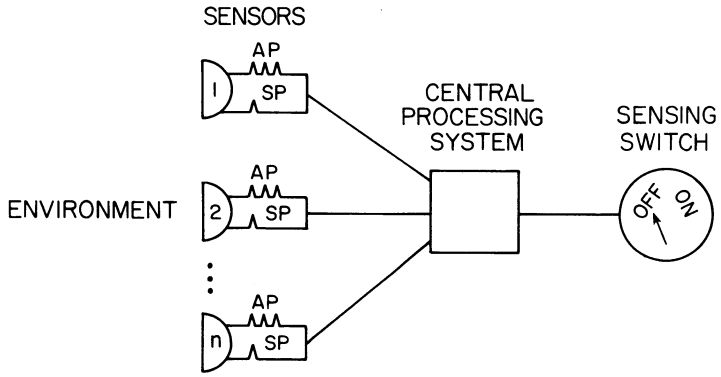

FIG. 6. Model of the quantitative processing of a pheromone stimulus in the deutocerebrum. The sensors represent pheromone-sensitive olfactory receptor neurons, the central processing system is analogous to a part of the macroglomerulus that receives summed input, and the sensing switch represents an indicator of a criterion behavioral response such as a reflex.

i.e., antennal movement or wing flutter (sections 4.6 and 6.2). In the figure these responses can be represented by the output sensing switch. However, the neurological responses that elicit and control the more complex orienting and mating behavioral sequences undoubtedly involve numerous complex processing and integrative steps in the mushroom bodies, the protocerebral lobes, and ultimately in the suboesophageal ganglion and segmental ganglia. Quantifying these latter processing steps, involving both pheromonal discrimination and integration with other sensory modalities, is an almost hopeless task at present, but some qualitative aspects of such interactions are discussed in the next section.

5 DISCRIMINATION OF PHEROMONE

For simplicity we have been treating the detection and quantification of a pheromonal stimulus as if the pheromone were a single chemical, neglecting the processes by which different pheromonal blends are discriminated. Such processes occur both at the peripheral level and at the level of the CNS. Most of the studies of pheromonal discrimination have been done at the periphery so discrimination by the CNS will be treated only briefly here.

The central processing of the pheromonal stimulus has not been investigated in detail, except for the studies of Boeckh, J. *et al.* (1976), Boeckh, J. and Boeckh, V. (1979), and Hildebrand, J. *et al.* (1980) showing that the macroglomeruli of the deutocerebrum appear to be the terminals of the axons of the pheromonal receptor cells. However, other studies of the responses by the CNS to food and general odorants provide additional insight into the mechanisms of pheromonal discrimination and the integration of the pheromonal response with other olfactory inputs as well as with other sensory modalities. Some processing appears to occur very early because neural responses to food odors in the deutocerebrum had a broader spectrum than the responses of the individual olfactory cells on the antenna (Waldow, U. 1975). Waldow found in *Periplaneta americana* that some neurons in the deutocerebrum were multimodal, as was also demonstrated by Masson, C. and Strambi, C. (1977). Selzer, R. (1979) recorded slow potentials possibly from interglomerular interneurons in response to food odors. The responses of these interneurons may be analogous to the responses of mitral cells in the vertebrate olfactory bulb which respond without impulses (Shepherd, G. 1981). Further processing of olfactory responses in the calyx, the α and β lobes of the mushroom bodies, and the protocerebrum (see Fig. 4) has been inferred from the recordings of cells within these neuropilar masses in *Apis mellifera* (Suzuki, H. and Tateda, H. 1974; Suzuki, H. 1975a,b; Suzuki, H. *et al.*, 1976). It should be noted that these responses would not be expected to feed back and influence the peripheral olfactory receptor cells (see, e.g., Payne, T. 1969; Payne, T. *et al.*, 1970). Integration of the olfactory response with other modalities occurs in these areas. Erber, J. (1978) found that 60% of the interneurons

in the mushroom bodies in *Apis mellifera* were multimodal. Some interneurons increased their sensitivity after multiple stimuli. It appears from the Erber investigation that discrimination in the higher centers of the CNS will be difficult to study.

In the remainder of this section we discuss primarily how pheromone is discriminated at the periphery, although there is some discussion of bioassays that undoubtedly reflect discrimination processes in the CNS. Aspects of discrimination are also discussed in two later sections, one dealing with the electroantennogram (section 6.1), and the other with behavioral correlates of the neural response (section 6.2). We examine several specific cases of pheromonal discrimination in Lepidoptera and Coleoptera, discussing the concepts of labeled lines *vs.* across-fiber patterning, specialist *vs.* generalist cells, and synergism *vs.* inhibition. From these examples we deduce some possible generalities before proceeding to a treatment of structure–activity relationships.

5.1 Discrimination by Lepidoptera

The earliest and most thorough of the studies of discriminatory processes in an insect was done with *Bombyx mori*. The work began when Schwinck, I. (1955, 1956) identified several hierarchical behaviors associated with pheromone perception. From these studies and the extensive neurophysiological studies of Dietrich Schneider and his colleagues at the Siewiesen Laboratories of the Max-Planck-Institute came the first concepts of pheromone discrimination in insects. In addition to discrimination in *B. mori*, we will consider in this section the discrimination of pheromones in *Trichoplusia ni*, *Argyrotaenia velutinana*, *Spodoptera litura* (F.), *Adoxophyes orana* (F.v.R.), *Lymantria dispar* and several *Yponomeuta* spp.

5.1.1 Discrimination by *Bombyx mori*

Boeckh, J. *et al.* (1965) introduced the concepts of specialist *vs.* generalist receptor cells and labeled lines *vs.* across-fiber patterning from interpretation of the electrophysiological responses in *B. mori*. They defined odor generalists as cells with unique and stable odor spectra that overlap the spectra of other generalist cells. Odor specialists were defined as cells that give stereotypical responses to a series of compounds. The latter group was exemplified by the pheromone-receptive cells. Nowhere were the specialist cells described as being sensitive to a *unique* chemical. Further definition of the specialist cell in *B. mori* was provided by Priesner, E. (1969) when he reported that "... 500 individual pheromone receptor cells investigated had the identical reaction spectrum". He also reported that all substances that activated the bombykol receptor cell in whatever concentration also elicited the criterion sexual behavior response. Also, the difference between the thresholds for a noticeable electrophysiological response was reflected in the differences between the behavioral thresholds. These observations are consistent with the definition of a "labeled line" for quality coding. In such a system the presence of activity in a specialist cell codes the odor quality (Mountcastle, V. 1968). The alternative would be coding by an across-fiber pattern of inputs from a number of cells with overlapping spectra. The simple labeled line concept of pheromone discrimination, coupled with early misconceptions of single chemical pheromones, has prevailed in some quarters until now.

Recent investigations, identifying other pheromone components (Kasang, G. *et al.*, 1978a,b) indicate that some of these mechanisms are not so simple even in *B. mori*. A second cell within the same sensillum trichodeum housing the cell responsive to bombykol has recently been found to respond to (*E,Z*)-10,12-hexadecadien-1-al (bombykal) (Kaissling, K. *et al.*, 1978), the bombykol cell responding also at high concentrations. These new discoveries revealed that discriminatory behavior in *B. mori* is clearly more complex than first believed. Bombykal alone is able to elicit some of the component sexual behaviors at concentrations 10^4 times that of bombykol, but paradoxically, it suppresses responsiveness when mixed with bombykol. For the present the encoding of odor quality in *B. mori* appears consistent with "across-fiber" patterns, with the effect of the aldehyde centrally determined.

5.1.2. Discrimination by *Trichoplusia ni*

In the two other species for which single olfactory cell responses, behavior, and pheromone chemistry

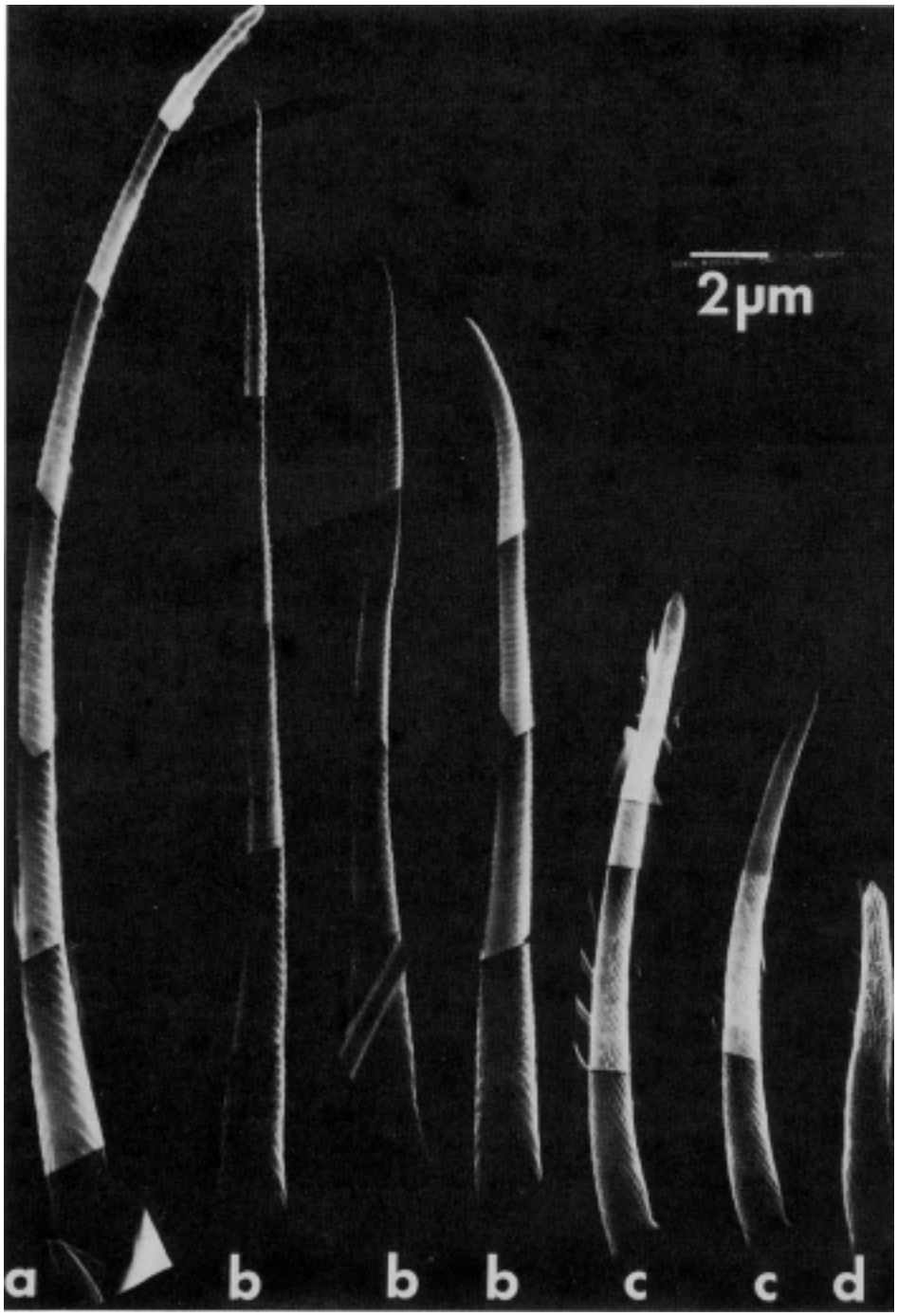

FIG. 7. Scanning electron micrographic collage of sendilla trichodea from *Trichoplusia ni*: (**a**) type ILS possibly containing the LS receptor cells, (**b**) either variants of type IHS possibly containing the HS receptor cell, variants of typr II, or three distinct additional types, (**c**) type II, (**d**) type III. Nomenclature of Mayer, M. *et al*. (1981). (photograph courtesy of R. J. O'Connell, Worcester Foundation.)

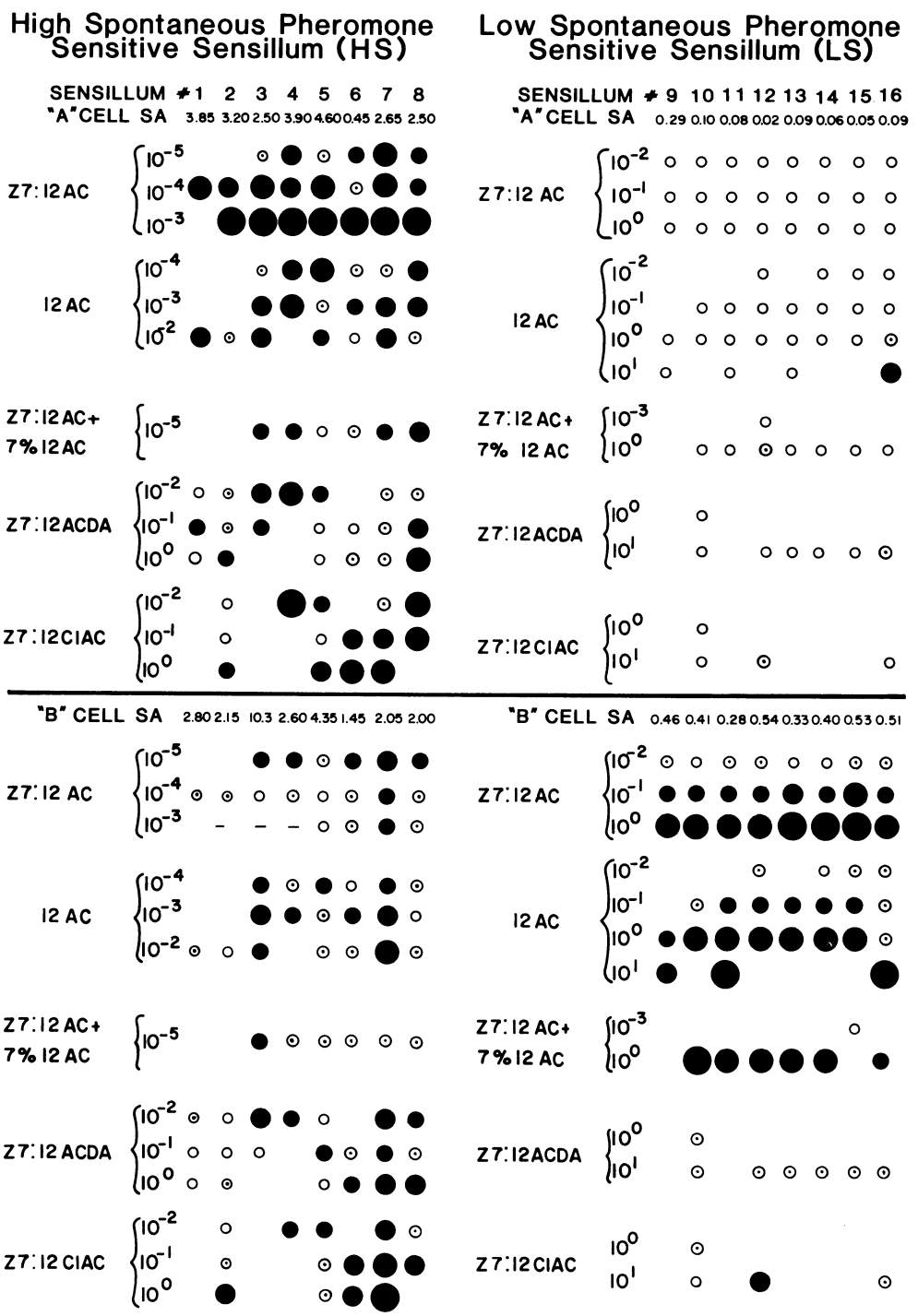

FIG. 8. Neurophysiological differences between two types of olfactory receptor cells ("A" and "B") within sensillar types designated as high-spontaneous activity pheromone-sensitive sensillum (HS) and low-spontaneous activity pheromone-sensitive sensillum (LS). The responses in number of impulses above spontaneous activity (SA) are coded as follows: ○, tested, no response; ⊙, 1–3; ●, 4–15; ●, 16–40; ●, >40. The chemicals are abbreviated as follows: Z7:12AC = (*Z*)-7-dodecen-1-ol acetate; 12AC = dodecan-1-ol acetate; Z7:12ACDA = (*Z*)-7-dodecen-1-ol diazoacetate; and Z7:12C1AC = (*Z*)-7-dodecen-1-ol chloroacetate.

are known, *Trichoplusia ni* and *Argyrotacnia velutinana*, there are similarities and dissimilarities in the peripheral encoding of the pheromone components and their blends. In *T. ni* there are sensilla trichodea containing receptor cells of at least two neurophysiological types that possibly can be distinguished by morphological differences (Fig. 7). Each of these two types contains two olfactory receptor cells differentiated collectively by their response spectra, "spike" amplitude, and spontaneous activity. A third cell of unknown functionality may also be present. The pheromone emitted by the calling female *T. ni* is a 93 : 7 mixture of (*Z*)-7-dodecen-1-ol acetate (Z7 : 12AC) and dodecan-1-ol acetate (12AC) (Bjostad, L. *et al.*, 1980b). Inspection of the responses and spontaneous activities in the two groups of cells reveals the existence of a more complex system of quality and quantity coding than is found in *B. mori* (Fig. 8). It is clear that the cell groups HSA and LSB are capable of coding a dynamic range of pheromone concentration of over seven orders of magnitude because of the quantitative differences in their sensitivity. Also, because these cells *always* responded to some concentration of both pheromone components they are clearly identified as specialist cells. It may be more accurate to further add a qualification to the definition of Boeckh, J. *et al.* (1965) that such cells respond most often and with greatest sensitivity to the major pheromone components at a physiologically significant range of concentrations.

Finally, as a further complication, another type of spontaneous cellular activity has been recorded in otherwise morphologically similar sensilla. The activity is characterized by one or more cells having a relatively high spontaneous activity and little separation of spike amplitude among cells in the same sensillum. No attempts have been made as yet to characterize the response spectra of the cells in these sensilla.

It is difficult at this time to relate the peripheral coding of quality to the behavior of *Trichoplusia ni*. Mixtures of Z7 : 12AC with 7% 12AC enhanced close-range behaviors (Linn, C. and Gaston, L. 1981). The enhanced attraction is possibly indicative of a synergistic process in which two pheromone components together produce a response that is greater than the sum of the responses to each component separately. Because the synergism could occur either at the periphery (as found in *Argyrotaenia velutinana* by O'Connell, R. 1972) or in the CNS, the pheromone-sensitive cells were stimulated with aliquots of 7% 12 : AC in Z7 : 12AC at appropriate concentrations in electrophysiological tests. No synergism was detected in these tests; consequently, the increased behavioral response in this case is probably a reflection of CNS processes.

Because both components of the pheromone are detected by the HSA and HSB cells, the HSB cell may be complementary in providing an across-fiber coding pattern. Some evidence for this is that the peripheral cells responded to (*Z*)-7-dodecen-1-ol chloroacetate but this did not result in an observable behavior in a small number of tests in a flight tunnel, nor in these tests did this chemical affect the response to Z7 : 12AC. Consequently, it appears that a labeled line of pheromone-sensitive cells does not exist in *T. ni*, even though these cells are specialists. Otherwise the insect would respond behaviorally to the chloroacetate.

Prior to further discussion of peripheral discrimination, we must assess the implications suggested by the two morphologically distinct types of sensillum trichodeum that house two neurophysiologically distinct families of pheromone-sensitive receptor cells. For example, are distinct morphological and neurophysiological types of pheromone-sensitive sensilla universal and, if so, does this assist or discommode interpretation of extant data? As to the universality, O'Connell (unpublished) has determined that there are two distinct morphological types of pheromone-sensitive sensilla in *A. velutinana* and *Lymantria dispar*, and that the two types in the former species are neurophysiologically dissimilar. No data on other insects are available.

As to the interpretation of extant data, it appears that the presence of two or more neurophysiological types of pheromone receptor cells is a complication for the neurophysiologist. The number of cells that must be sampled increases, and the number of chemicals and the permutations of their mixtures become unwieldy. Thus, the study of quality coding at the periphery is expected to be slow, and understanding of even a few principles for a few insects will be difficult. No unifying concepts of peripheral odor coding exist—perhaps none are possible.

In one sense, the existence of two or more

neurophysiological types of pheromone receptor cells may simplify the interpretation of some electrophysiological data. For example, Priesner, E. (1980) believes that there are two to five receptor-cell types within some chemosensilla of *Ochropleura plecta* (L.) and *Cacoecimorpha pronubana* Hübner. None of the cells from the two different species of insect responded consistently to any chemical tested. In particular, there was a lack of consistent response by the four cells of *C. pronubana* to (*Z*)- and (*E*)-11-tetradecen-1-ol and (*Z*)- and (*E*)-11-tetradecen-1-ol acetate (Z11:14AC, E11:14AC), components of the pheromone blend of this species (Descoins, C. and Frerot, B. 1979). Perhaps this lack of consistency is a reflection of the existence of cell types that have not yet been distinguished electrophysiologically or morphologically.

5.1.3. Discrimination by *Argyrotaenia velutinana*

Another study of the peripheral cells that respond to the sex pheromone is that of O'Connell, R. (1972, 1975) on *Argyrotaenia velutinana*. This is a tortricid whose sexual behavior is governed by a complex blend of 12AC, Z11:14AC, and E11:14AC (Roelofs, W. *et al.*, 1975; Baker, T. *et al.*, 1976). O'Connell, R. (1972) recorded the activity from two cells that responded to the three components of the pheromone. These cells do not appear to exhibit the extreme sensitivity shown by the HSA cells in *Trichoplusia ni* or cells in *Bombyx mori* studied by Kaissling, K. and Priesner, E. (1970). Both of the cells studied by O'Connell, R. (1972) exhibited a synergistic response to mixtures of Z11:14AC with 12AC. Also, both cells were inhibited by mixtures with the geometric isomer.

Quality coding of pheromone by individual olfactory receptor cells of *A. velutinana* more resembles the system of *T. ni* than it does *B. mori*. In the former two species the coding is clearly an across-fiber system in which (probably) two cells have an identical response spectrum to any particular odorant. This also appears to be the case for the long sensilla trichodea of *Antheraea pernyi* for odorants other than the pheromone (Schneider, D. *et al.*, 1964). Probably, the greatest sensitivity of this type of specialist cell in *A. velutinana* is to the major pheromone components, although not all the cells can be expected to respond even to these compounds.

5.1.4 Discrimination in other Lepidoptera

Recordings from the receptor cells of several other species of Lepidoptera provide further insight into peripheral encoding of pheromone quality and quantity. The recordings of van der Pers, J. (1982) demonstrates the existence of high and low spontaneously active cells in different *Yponomeuta* species which were responsive to different pheromone components. Barybkina, M. (1980) recorded from cells having differential sensitivity to the pheromone and its analogs. These two investigations suggest that discrimination in these insects is similar to the system described for *Trichoplusia ni*.

Two other species, *Spodoptera litura* and *Adoxophyes orana* are trapped predominantly only by mixtures of two of their component pheromone isomers. Although requisite morphological and neurophysiological details are not available for these two species, it appears that the behavioral synergy is reflected, at least partly, in the responses of the peripheral receptor cells. The sensitivity of cells of *S. litura* studied by Aihara, Y. and Shibuya, T. (1977) resemble the sensitivity of HS cells in *T. ni*. However, unlike in *T. ni*, the cells studied responded to one pheromone component, (*Z,E*)-9,11-tetradecadien-1-ol acetate, at the lowest concentration, but not to the other, (*Z,E*)-9,12-tetradecadien-1-ol acetate. A 9:1 mixture of the former with the latter compound elicited the greatest rate of action potentials, other ratios eliciting much less activity.

A similar, but not identical, synergism was observed by Den Otter, C. (1977) in *A. orana*. In this species the sensitivity of the cells studied resembled the LS cells of *T. ni* and the response by the "A" and "B" cells to Z11:14AC and E11:14AC closely paralleled the responses observed by O'Connell, R. (1972, 1975) in *A. velutinana*, also a tortricid. In the case of *A. orana*, however, no synergism as dramatic as that of *S. litura* was observed in the peripheral cells to mixtures of the two pheromone components. Clearly, until the presence or absence of the types of cell lines demonstrated in *T. ni* are established for other species, the mechanisms of discrimination and synergism must remain shrouded in mystery.

5.2 Discrimination by Coleoptera

The details of discrimination by olfactory cells of *Ips pini* (Say) and *Ips paraconfusus*, respectively, are discussed at length by Mustaparta, H. *et al.* (1979) and Mustaparta, H. (1979, 1980). Correlation of the responses of individual olfactory cells with behavior is difficult, and probably would be more satisfying if all the cells capable of responding to the pheromones were completely classified. Also, the CNS appears to be entirely responsible for behaviorally observed synergism and inhibition because no synergism was observed at the periphery. From the viewpoint of olfactory transduction, it is interesting that individual peripheral cells can discriminate quantitatively between pheromone enantiomers (see section 4.3.2); these cell lines furthermore respond exclusively to either ipsenol or ipsdienol, which are possibly used for interspecific sexual isolation. In fact, Mustaparta suspected that the cells could be further subdivided into families that discriminate between host- and insect-produced odors.

There is thus no question that the olfactory physiology of beetles is much more complex than in moths. The sexual behavior is very complicated, and is strongly affected by chemicals from host trees as well as by mixtures of chemicals produced by both sexes of the same species and of related species. Furthermore, the behavior can be modified by stridulation. Nonetheless there appears to be an overall similarity in the peripheral olfactory cells of the beetles and those of the Lepidoptera. These similarities are the previously discussed combination of different types of specialist cell having concomitant subdivisions and the generalist cells having broad response spectra. The response spectra of the latter do not include the pheromone or host-odor component chemicals at the concentrations tested (Mustaparta, H. 1975; Mustaparta, H. *et al.*, 1979).

5.3 Structure–activity relationships

Behavioral and electrophysiological studies of discrimination have often been conducted to obtain knowledge about putative receptor–site interactions. Usually, differences in the effects of small structural changes in the stimulus molecule on some known biological response or biochemical reaction are compared. However, in attempting to analyze structure–activity relationships via behavioral responses, an immediate difficulty becomes evident with pheromones. Usually, stimulation by a specific blend of chemicals is required to produce a specific criterion behavioral response. Use of blends of even two chemicals enormously complicates the evaluation of the effects of structural changes, and the use of three appears doomed even under the best of assay methods. Ultimately, bioassay responses are only indirectly reflective of pheromonal interactions with receptor sites. In section 4.6 we showed that there is much intervening neural processing between the response of peripheral receptor cells and the behavioral response, which makes obsolete the idea that field trapping, or even active flying, as well as most other behavioral assays, can be a reliable measure of receptor site interactions. Essentially, no behavioral assay is capable of distinguishing between a failure by an odorant to induce some criterion behavioral response and a lack of receptor cell response to the odorant.

Discrimination in single olfactory receptor cells can be studied by standard electrophysiological procedures. Structure–activity relationships can be established from the particular type of cell under scrutiny. If only one type of receptor cell is present on the antenna, as apparently is the case for *Bombyx mori* and *Antheraea pernyi*, the EAG also can be used for structure–activity studies. However, for insects such as *Trichoplusia ni* and *Argyrotaenia velutinana*, where single cell and scanning electron microscopical techniques have shown that more than one cell type is present, the EAG, being a summation response of all the cell types, would not appropriately measure the discriminative capacity of an individual cell type.

Structure–activity correlations (molecular discrimination) are important measures for pharmacological receptor interactions, the concept of transduction currently in favor. The actual number of different proteins that interact with pheromone components is not known. We can speculate that if each cell in the proper sensillum has only one different protein receptor, then, for example, *B. mori* may have only two types. But for *T. ni* and *A. velutinana*, there are two or three classes of sensillum each having two or three cells that

respond differentially to pheromone components. We could assume that there are as many as six different receptor proteins in *T. ni* receptor cells. Other alternatives are possible, such as a differential response due to the effect of sensillar morphology on diffusion. Also, the interactions between the receptor potential and the spike generator in different cells could modify the interactions of a single receptor molecule. Presently, no methodology is available to assess all these possibilities. Perhaps a variant of the usual EAG stimulation procedure resulting in what is termed "differential adaptation" (Payne, T. and Dickens, J. 1976; see also Payne, T. and Finn, W. 1977) can be used in certain circumstances to obtain a measure of protein–odorant interactions. Also, the EAG can be used to show that insects can discriminate between enantiomers (Bestmann, H. *et al.*, 1980). In other cases, a bioassay can yield useful information as long as it measures the presence, rather than the absence, of discrimination. Bioassays have shown that there are two stereospecific chemoreceptors for an achiral pheromone in *A. velutinana* (Chapman, O. *et al.*, 1978), as well as for chiral pheromones in a few beetles such as *Popillia japonica* Newman (Tumlinson, J. *et al.*, 1977). However, none of these correlates of protein–odorant binding provides an unambiguous assay of the fundamental cellular response. All have been used in the past with varying imprecision. To understand what can be done with these correlates requires a thorough understanding of what they are, and how they may be affected by uncontrollable factors.

6 CORRELATES OF THE NEUROPHYSIOLOGICAL RESPONSE

Besides the measurement of membrane potential and spike activity in single olfactory receptor cells, there are two other correlative methods for obtaining information about an insect's response to sex pheromone, as well as gaining an insight into the functioning of the CNS: the EAG and the bioassay of a criterion behavioral response, e.g., wing flutter or flight responses. The EAG measures the potential difference induced between the distal and proximal tips of an antenna during an odorant stimulus. This potential difference is proportional to the sum of the generator potentials of all the stimulated sensory cells on the antenna (Schneider, D. 1962; Mayer, M. *et al.*, 1982). Thus the single cell and EAG responses are correlated.

In contrast, the correlation of discriminatory behavior to either single cell or EAG responses is limited. Even in the simplest case of a simple reflexive response to sex pheromone, cues from non-olfactory stimuli are processed and integrated by the central nervous system prior to induction of the behavioral response. Consequently, a bioassay response does not necessarily reflect solely the reaction to pheromone. While the bioassay is often a useful tool for determining when and if an insect perceives pheromone, the failure to respond behaviorally cannot be used as a determinant of the discriminatory range of a receptor cell to groups of odorants.

In the following subsections we examine the physiological basis of the EAG, as well as adaptation effects and the use of the EAG to study discrimination. Then we consider the linkage between olfactory perception and behavior, including how some genetic alterations of behavior possibly are linked to changes in the peripheral pheromone receptor cells. The quantification of the behavioral response, habituation, inhibition, synergism, and other modifications of the behavioral response to pheromone also are discussed.

6.1 The electroantennogram

Dietrich Schneider (1955, 1957) was the first to record and correctly interpret EAG responses from insect antennae exposed to pheromone. Earlier attempts to record spike activity and slow potentials from the antenna by Chapman, J. and Craig, R. (1953), Roys, C. (1953), Boistel, J. (1953), Boistel, J. and Coraboeuf, E. (1953), Boistel, J. *et al.* (1956), and Uchiyama, H. and Katsuki, Y. (1956) were not entirely successful. The difficulties that Schneider encountered in measuring the antennal response cannot be fully appreciated by those who now have more than 20 years of experience with the technique as well as more refined basic information pertaining to the EAG.

6.1.1 Physiological basis of the EAG

At first, Schneider likened the EAG to the electroretinogram, but by 1962 he had proposed the

EAG to be the "... summed recording of the activity of many receptors ...". He was skeptical of the quantitative validity of the EAG until he and his colleagues obtained a standard sigmoidal response curve over a range of doses from about 10^5 to 10^{10} molecules cm^{-3} (Schneider, D. *et al.*, 1967). The demonstration of this summation relationship depended on detailed morphometric analyses of the various antennal sensilla of *Trichoplusia ni* (Fig. 9). Two methods of recording EAGs from specified numbers of sensilla were used: one that required progressive excision of antennal subsegments on the same antenna, and another that confined the stimulus to localized subsegments along the antenna. These data and interpretations were supported earlier by more limited experiments conducted on *T. ni* by Payne, T. (1969) and by Roelofs, W. and Comeau, A. (1971b) with *Argyrotaenia velutinana*, Behan, M. and Schoonhoven, L. (1978) with *Pieris brassicae* L., and Nishino, C. and Takayanagi, H. (1979) with *Periplaneta americana* L.

In determining the contribution to the EAG response that is due solely to pheromone, it is necessary to account for the incidental activity from receptors for non-pheromonal odorants and other sensory modalities. This incidental activity can constitute a sizeable fraction of the response (Schneider, D. *et al.*, 1967; Payne, T. *et al.*, 1970; Grant, G. 1970; Minks, A. *et al.*, 1974; and others), or it can be very small or absent (Behan, M. and Schoonhoven, L. 1978; Angst, M. and Lanier, G. 1979; and others). The usual consensus of investigators has been that

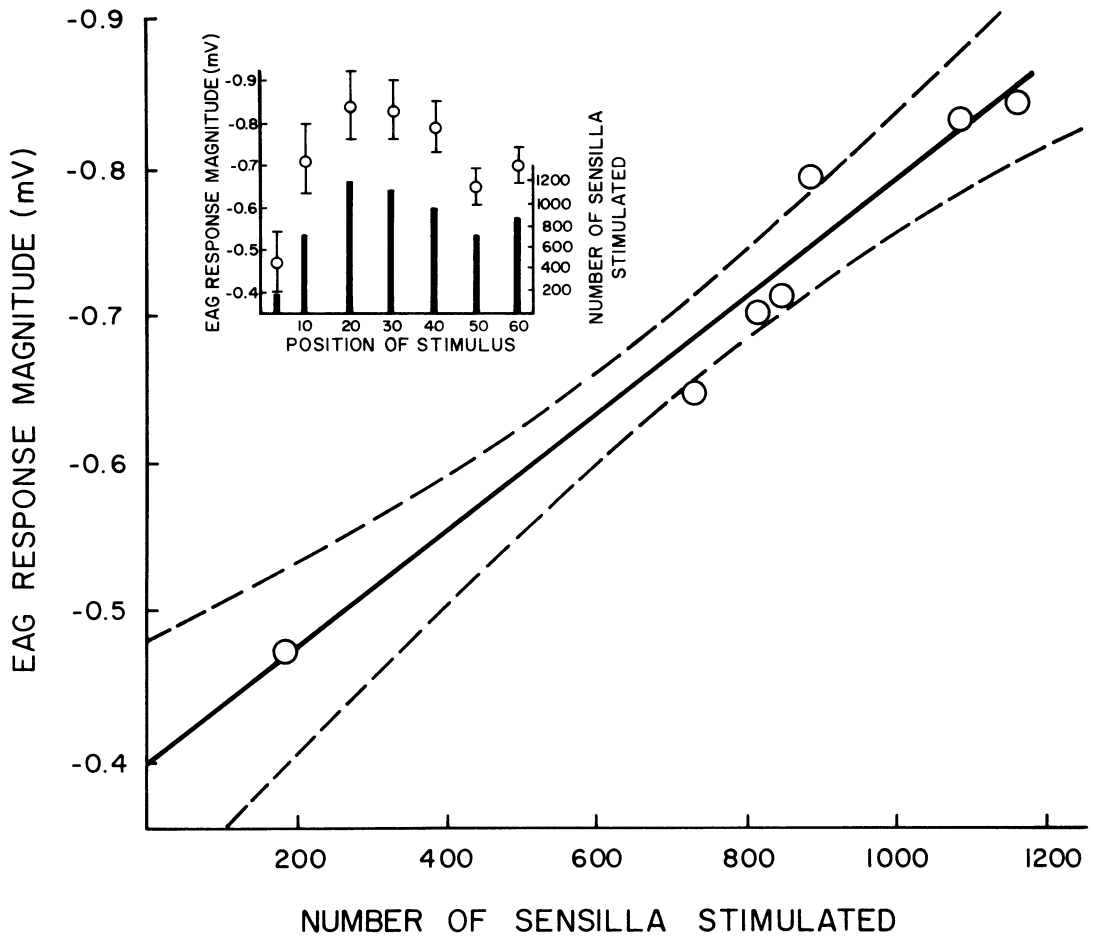

FIG. 9. Correlation of EAG magnitude with the number of sensilla stimulated using a technique that confined the stimulus to specific subsegments. The inset shows the response to stimuli centered at the specific subsegment number. The main graph was obtained by determining the number of sensilla subtended by the stimulus.

the background response is the result of unavoidable temperature and moisture fluctuations, as well as the effects of mechanical stimuli. Heretofore, the background response has been considered to be a "blank" and consequently simply subtractable. However, the magnitude of the EAG response to pheromone plus background was shown by Mayer, M. (unpublished) to be non-linearly affected by the background response at high concentrations of pheromone, and thus cannot merely be subtracted.

Because the background response so confounded interpretation of the EAG response to pheromone, Mayer, M. (unpublished) intensively investigated procedures for eliminating "blanks". They found that the only reliable way to eliminate contaminants from the room air was to continually pass a clean carrier air stream over the antenna. Once the room air contaminants were eliminated, a further source of background responses was observed. This was identified as a plasticizer that accumulates in air standing in the Teflon® tubing, and it was eliminated by purging all parts of the stimulus delivery system immediately prior to testing. As a consequence of identifying and eliminating these sources of contamination, a zero background response was obtained for *T. ni*, and an enhanced sensitivity of the EAG to pheromones was observed.

Some investigators hypothesize that certain physiological factors affecting behavior are reflected by changes in the EAG. However, the EAG, because it is a measure of the summed responses of peripheral cells, would not likely be influenced by time-of-day phenomena or circadian rhythms, because these effects probably originate in the CNS. Likewise, age would not be expected to directly affect the EAG once development of the olfactory cells is complete, approximately at eclosion (Schweitzer, E. *et al.*, 1976). Payne, T. (1969) showed that the time of day did not affect the EAG response in *T. ni*. Some undescribed physiological phenomena do influence the EAG, manifesting themselves in the variability of responses among individual insects (Roelofs, W. and Comeau, A. 1971b; Mayer, M., unpublished).

6.1.2 Adaptation in the EAG

The EAG exhibits adaptation phenomena that become more pronounced as the odorant concentration increases (Boeckh, J. *et al.*, 1965; Schneider, D. *et al.*, 1967; Grant, G. 1970; Kaissling, K. 1977a). The adaptation can last from a few seconds to hours (Boeckh, J. *et al.*, 1965; Payne, T. 1969; Kaissling, K. 1975, 1977a,b).

The time course of EAG adaptation has not been studied intensively, partly because so many mutually interacting parameters are involved, including:

(1) adapting concentration;
(2) length of adapting interval;
(3) length of recovery interval; and
(4) stimulus concentration.

Differential adaptability of various chemicals is another obvious interactive factor (section 6.1.3). Four of these factors have been investigated briefly by Kaissling, K. (1975, 1977a,b) with *Bombyx mori*. Seabrook, W. (1977) investigated effects of concentration and recovery interval in *Choristoneura fumiferana* (Clemens). The effect of stimuli delivered over a long time course on *Ostrinia nubilalis* was studied by Nagai, T. *et al.* (1977). Repetitive stimuli of 10 μg loads of Z11 : 14AC or E11 : 14AC from filter paper were cross-adaptive, and adaptation apparently persisted for about 20 min. Intervals of 10 to 15 s between repetitive stimuli at low pheromone concentrations did not result in measurable adaptation in *T. ni* (Mayer, M. 1973). In the above lepidopterans and in *Dendroctonus frontalis* Zimmermann (Payne, T. and Dickens, J. 1976), pheromonal adaptation to high concentrations is rapid, but there is a return of sensitivity within minutes, although in *B. mori* complete disadaptation from a strong stimulus required hours. Unfortunately, behavioral comparisons with the EAG appear impossible because of the rapid and long-lasting habituation effects in behavior that are centrally mediated (see section 6.2.3).

The physiological basis of the EAG adaptation is the adaptation of the generator potential of single olfactory receptor cells. Kaissling, K. (1977a) enumerated several potential origins of individual receptor cell adaptation, including:

(1) a lowered affinity of binding to the receptor molecule;
(2) a decreased rate of its activation; and
(3) a reduction in the change of membrane conductance triggered by the activated receptor molecule.

The difficulty of explaining EAG adaptation in terms of receptor cell response adaptation is exemplified by the findings that various parts of the receptor cell can adapt differently because the nerve impulses often adapt much sooner than the receptor potential, and that impulses from cells of the same sensillum show evidence that the cells adapt independently (Kaissling, K. 1977a). It appears that prior to assembling a unified theory of the EAG, the responses of individual receptor cells must be determined with greater precision, and a method of extrapolating these data to predict the response of the entire ensemble of the antenna must be derived.

6.1.3 Discrimination and the EAG

Notwithstanding the difficulties mentioned above, many of the discriminative and adaptive characteristics of groups of olfactory receptor cells can be studied quickly by use of the EAG. Often the EAG response is correlated to the behavior of the insect as well. For example, the EAG appears to respond with the greatest amplitude to the chemical eliciting the greatest behavioral response (Schneider, D. *et al.*, 1967; Payne, T. 1969, published in Gaston, L. *et al.*, 1972; Roelofs, W. *et al.*, 1971; Priesner, E. 1979; and others). Differential sensitivity to various binary mixtures has been demonstrated, although the statistical significance of the differences is not overwhelming. Minks, A. *et al.* (1974) showed that *Clepsis spectrana* (Treitschke), but not *Adoxophyes orana*, responded differentially to binary mixtures of two-positional isomers of a monounsaturated 14-carbon acetate at low concentrations. Higher concentrations of binary mixtures of geometrical isomers may be required to show differential sensitivity in *Argyrotaenia velutinana* (Baker, T. and Roelofs, W. 1976). EAGs elicited from *Trichoplusia ni* did not show clear differences in response to mixtures of the pheromone components, Z7 : 12AC and 12AC (Fig. 10). That the EAG responses to these mixtures exhibit differential sensitivity in some insects but not in others is possibly indicative of different mechanisms of discrimination among various insects (see section 5). It may also be argued that differences in recording characteristics of different insect antennae obscure any single-cell discrimination actually occurring in those insects where differential sensitivity in the EAG has not been observed.

"Differential adaptation" is a term first used by Payne, T. and Dickens, J. (1976) and Payne, T. and Finn, W. (1977) that refers to an EAG measurement paradigm they believe is capable of clarifying "acceptor" (receptor site) specificities. Differential adaptation of the EAG has been used in studies on *Dendroctonus frontalis* and *Galleria mellonella* in attempts to determine whether two chemicals stimulated identical or different classes of receptor cells. This variant in procedure was used to assess discrimination because the differences between the response of both male and female *D. frontalis* or *Dendroctonus brevicomis* LeConte to the primary pheromone component (frontalin) and to several terpenes that modulate sexual behaviour were slight (Payne, T. 1975). Repeated stimuli delivered within 10 ms of adaptation resulted in a much diminished EAG. However, some chemicals cross-reacted differentially in a manner that suggested a greater reaction of frontalin molecules with (presumably) single receptors or receptor sites than the behavior-modulating terpenes. A related study of cross-adaptation was performed by Kaissling, K. (1977a), who conditioned *B. mori* to high concentrations of a bombykol analog, (*E*)-10-hexadecen-1-ol. This analog did not cross-adapt the antenna to bombykol although the response to bombykol was self-adapting. In other studies Payne, T. and Dickens, J. (1976) and Dickens, J. and Payne, T. (1977) obtained evidence that adaptation occurred at the level of individual olfactory cells on the antenna, and concluded that more receptor sites reacted with frontalin than with the other chemicals.

Payne, T. and Dickens, J. (1976) interpreted the antennal responses they obtained as the interaction of the olfactory stimulants with one or more acceptors (= receptor sites) located on one physiologically distinct group of cells. We know now that there are more than one distinct physiological and perhaps morphological group of receptor cells that respond to any particular chemical or group of chemicals. Indeed, Payne, T. and Finn, W. (1977), expanding their studies with the differential adaptation technique to a lepidopteran, found they had to hypothesize the existence of two different receptor or "acceptor" sites on the pheromone receptor cell of *G. mellonella* to adequately explain the data in terms amenable to their concept of "acceptors".

Although Payne, T. and Dickens, J. (1976) quite

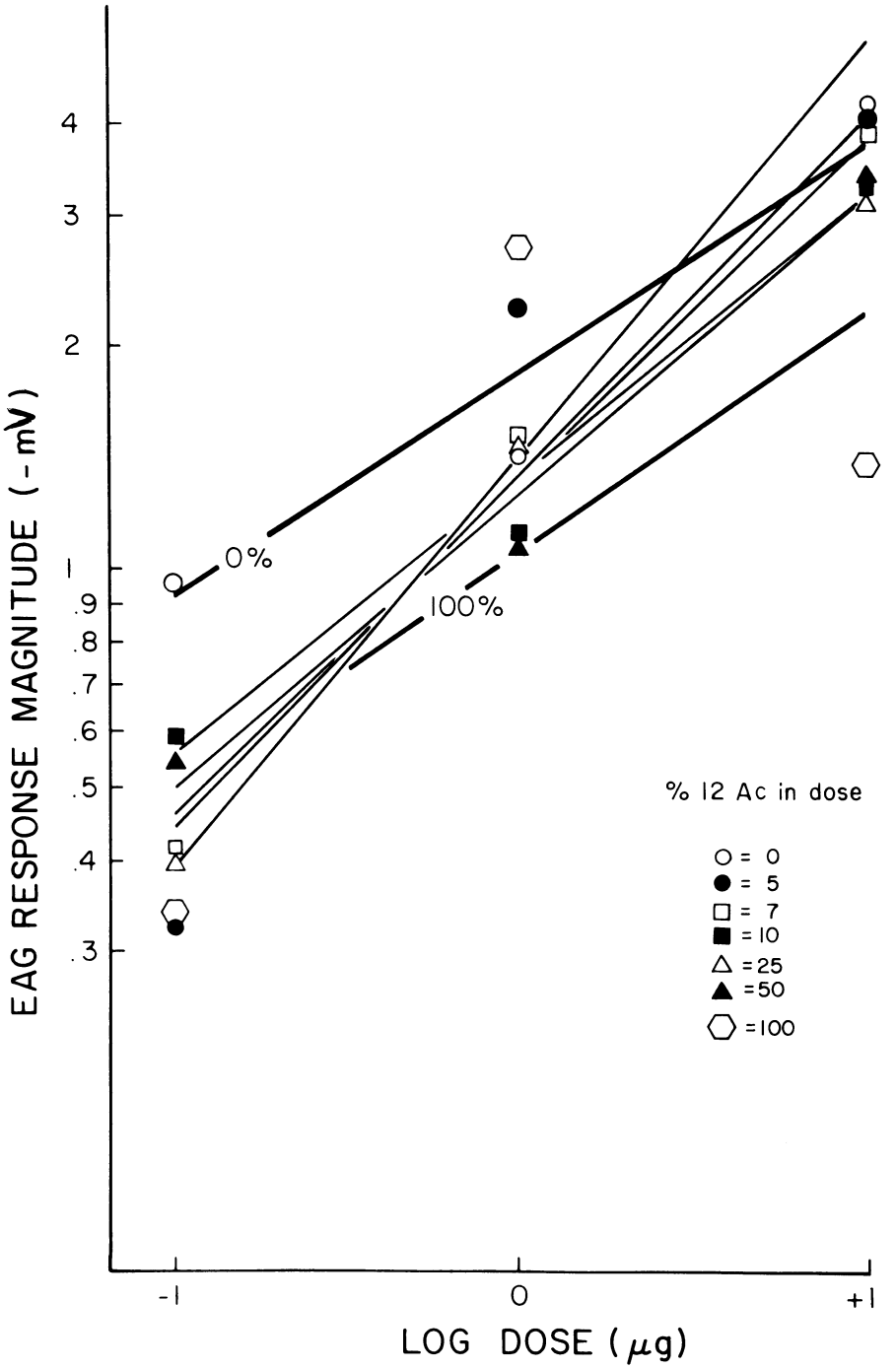

FIG. 10. EAG response of *Trichoplusia ni* to various mixtures of 12AC with Z7:12AC at three dispenser dosages.

correctly assumed that more than one kind of receptor sensitive to a particular chemical could be located within any one cell group, they did not know of the distinct groups of receptor cells. It is likely that summed responses of these groups of distinct cells can mimic some of these responses observed from EAG measurements of differential adaptation. Another complication arises if we

ponder the possibility that some of the physiological distinctiveness of cells within a cell group is a result of different receptor types as well as the existence of some interaction between the receptors and the structure of the sensilla. Because of such complications, other single cell techniques probably will prove to be more useful in the study of pheromone discrimination.

6.2 Behavioral correlates of neural responses

According to Kennedy, J. (1968) the analysis of behavior is a search for "... relations between neural mechanisms that produce the shifting singleness of action of the whole animal...". He also notes that the tools of the neurophysiologist are powerful but inappropriate, suggesting that "... reflex analysis of those relations provides the appropriate method. Until sufficient results are available from it the search for neural mechanisms must be blind." Such statements were accurate and penetrating assessments of the state-of-the-art in 1968. However, now there are enough data available, including the studies of pheromonal searching behavior by Kennedy, J. (e.g., 1980) himself, to provide a solid basis for neurophysiological investigations of some aspects of the pheromonal response in the CNS. The near reflexive nature of both the initial response to pheromone and the behavior at the margin of a pheromone plume make the neurophysiology of these behaviors particularly appropriate for analysis (see section 4.7).

Unfortunately, the current usage of definitions distinguishing reflexive, pheromone-induced behaviors is subject to imprecision. For example, "attraction" is a term commonly applied to the behavioral response to pheromone. But this is "... no more than a blanket teleological term signifying an end result, conveying nothing about the component stimuli or reactions" (Kennedy, J. 1978). To properly link behavior with neurophysiology we must formulate definitions of mutual concepts in terms compatible to both disciplines. Also, it is important to have some concept of the neurophysiological details that a specific behavior can or cannot identify and define. Several of the centrally mediated component behaviors that result in capture of insects in the field have been elegantly demonstrated for *Argyrotaenia velutinana* by Baker, T. *et al.* (1976). Such a sequence of behaviors to pheromonal stimulation also was demonstrated in *Bombyx mori* (Schwinck, I. 1954, 1955, 1956), and for *Trichoplusia ni* from the efforts of H. H. Shorey, his students, and colleagues (Shorey, H. 1977) (see also Bartell, R. 1977). As a consequence of the current imprecision and misunderstanding of definitions for pheromone-induced behavior, some investigators have incorrectly attempted to deduce structure–activity relationships using trapping, attraction, other non-definitive behaviors, and the EAG as definitive tests of olfactory detection. However, these measures of response are not appropriate for structure–activity studies of peripheral sensation (see section 5.3).

In the next subsection we reconsider what is known qualitatively about the linkage between the peripheral olfactory cells and the observed behavior. Afterwards we discuss the genetic alteration of this linkage and various modifications to pheromone-induced behavior, including habituation, adaptation, synergism, inhibition, and hormonal stimulation. The remainder of the section deals with quantitative measures of the behavioral response to pheromone.

6.2.1 The linkage between peripheral olfactory input and efferent reaction

The prevailing concept of pheromone-induced behavior is that it is "released" from some inhibited state. In this scheme release refers to activation of "prewired" reflexive feedback loops which, once activated, command the motor fibers that are responsible for the muscular co-ordination in the behavior (Fraenkel, G. and Gunn, D. 1961). The processes by which the feedback loops are activated are poorly understood. Howse, P. (1974) believes that the triggered sub-routines of insect reflexive behavior include an amalgam of interactions of the after-effects of one activity upon another. Miller, P. (1968) sums up the knowledge then and now, saying: "... on the motor side there is much information ... about activity in the final pathways ... but less about the mechanisms which order the activity".

A key problem in attempts to determine the neural pathways by which an odorant stimulus activates reflexive motor-fiber loops is that little is

known about the routing of olfactory information through the brain. In section 4.6 we noted that the primary route of information from the peripheral olfactory receptor cells to the higher centers of the brain extends through the antennal lobes and thence to the calyx of the mushroom bodies, from whence fibers also link the two lobes of the protocerebrum (Fig. 11). Strausfeld, N. (1976) also

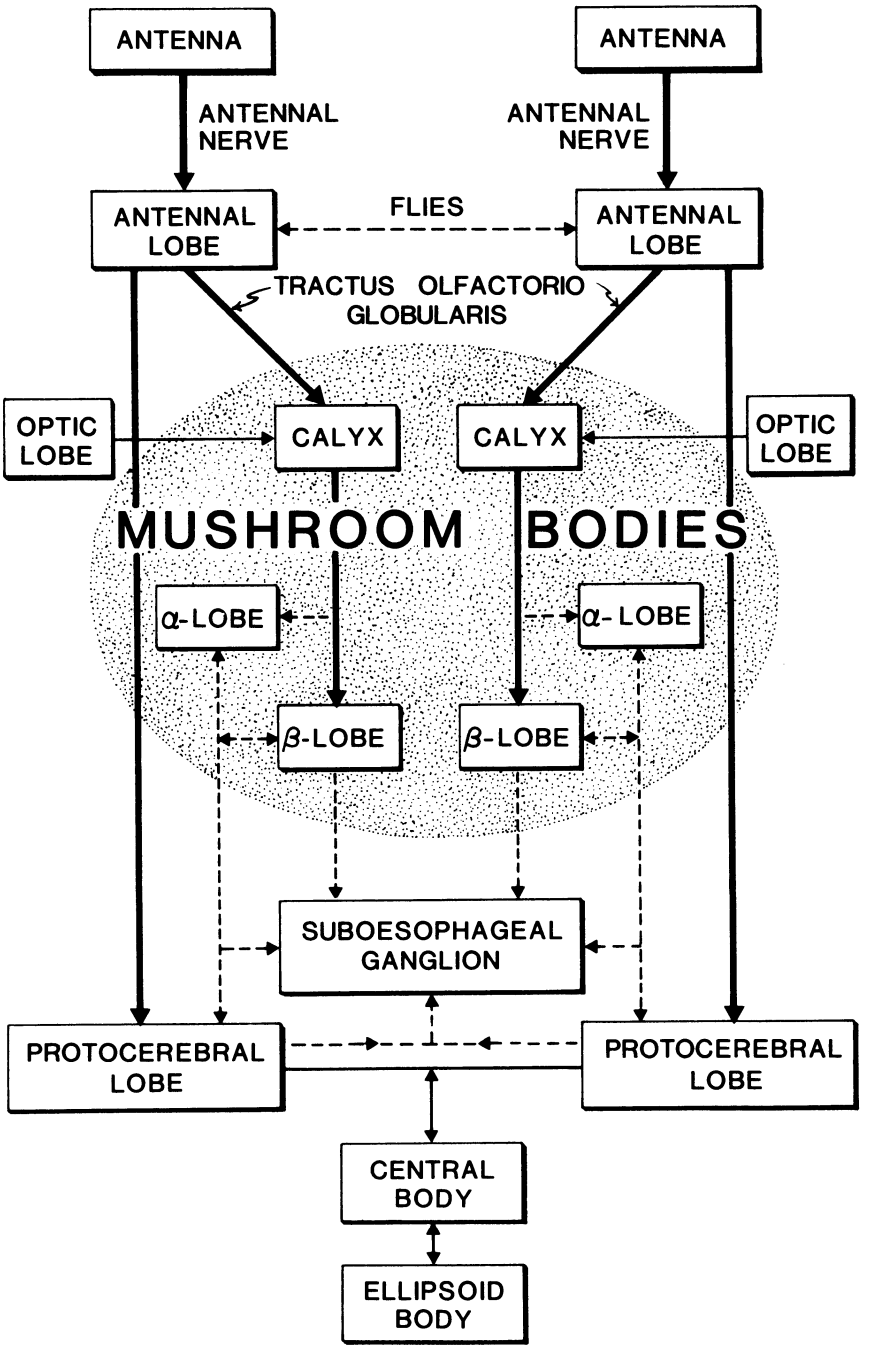

FIG. 11. Schematic diagram of the major neuropile areas of the insect brain. Heavy lines denote known routes of olfactory information flow; dashed lines denote conjectural routes.

identified some contralateral fibers between the antennal lobes in flies, but Boeckh, J. and Boeckh, V. (1979) saw no evidence of contralateral fibers among the pheromone-sensitive units they studied in *Antheraea pernyi* and *Antheraea polyphemus*. Some evidence exists that there are other routes of olfactory information through the brain (see also section 4.6).

It is thought that the central body couples and/or selects the various pieces of neural information for ultimate coupling with motor neurons to produce the appropriate behavioral response (Miller, P. 1968). However, the location and construction of the central body suggests more to Howse, P. (1974) that it has a generalized function such as arousal, and could thus affect such functions as behavioral threshold. Furthermore, Huber, F. (1965) reported that destruction of the central body depressed locomotion and that stimulation evoked fast running, jumping, and flying in *Gryllus campestris* (L.). Whatever the function of the central body, the recordings of olfactory responses by Mimura, K. *et al.* (1969) from the protocerebral lobes clearly implies that there is also some role of this neuropilar region in the activation of the behavioral response.

The pheromone-induced behaviors most amenable to study by neurophysiological techniques are the orientation behaviors, primarily osmoklinotaxis, osmotropotaxis, and anemotaxis. Osmoklinotaxis and osmotropotaxis occur in *Apis mellifera* (Markl, H. and Lindauer, M. 1965) and in *Drosophila melanogaster* Meigen (Borst, A. and Heisenberg, M. 1982). Osmoklinotaxis demonstrates a neurological process that allows the insect to detect differences in odorant intensity between two successive positions, which requires that the insect have a "memory". Osmotropotaxis demonstrates the ability to detect a spatial concentration gradient between two antennae. However, a pheromone plume quickly develops inhomogeneities in concentration as it travels downwind of the source because of the effects of turbulence. This inhomogeneity makes osmoklinotaxis and osmotropotaxis unreliable except at short distances. At longer distances the insect searches by more complicated mechanisms including anemomenotaxis (Kennedy, J. 1977, 1980).

Little is known about the location of the neurons that govern the orienting processes necessary for mate-hunting through upwind flight. However, the observation that an insect tends to maintain itself within the confines of an odorant plume suggests a means to study both the routes of further central processing of odorant-derived information and the location of the parts of the brain controlling such orientation. The keys to such studies may be the on–off and off–on signals critical to pheromonally induced anemotactic behavior (see section 3.3). Kennedy, J. (1980) demonstrated that for *Adoxophyes orana* the critical signal is the response onset, although we can adduce that alternative signals might be used by other species. In earlier studies the increase in the rate of turning by a moth when it flew outside the plume seemed to indicate the critical signal was an off-response. Of the major neuropilar areas the one most likely to control the steering commands is the protocerebrum. Roeder, K. (1937) reported that the protocerebrum steers locomotion in *Mantis religiosa* L. Similarly, Huber, F. (1965) found that turning commands governing leg movement appear to be generated in the protocerebral lobes. Howse, P. (1974) believes, furthermore, that the mushroom bodies inhibit locomotion and the central body counteracts the mushroom bodies.

Cognizant that some of the requisite nerve fibers have not been found, we conceptualize the following sequence of neural events for a moth lying upwind in a pheromone plume. First, feedback loops in the central body contribute to the alertness action patterns early in the behavioral hierarchy. Second, fibers projecting into the protocerebral lobes that control the turning behaviors are synchronized by off–on and/or on–off fibers monitoring the edges of the pheromone plume. We believe that the discrimination required for the more advanced behaviors occurs predominantly in the mushroom bodies for several reasons. First, most if not all the neurons from the deutocerebral glomeruli synapse at the calyx of the mushroom bodies. Second, responses of multimodal neurons appear to be integrated here. Finally, complex inhibitory functions appear to be associated with this neuropile as well as feedback loops (see section 4.6). Thus, it is likely that environmental influences on pheromone-induced behavior are integrated here. Ultimately, command fibers initiating the proper

behavior are somewhere connected to the suboesophageal ganglion.

Behavioral observations and trapping studies suggest that the discriminative criteria which must be satisfied to release a specific pheromone-induced behavior increase as the insect progresses along its hierarchy of pheromonal responses. An illustration of this process is found in a study of the responses of the moth, *Acrolepiopsis assectella* Zeller to its pheromone and analogs (see also section 6.2.4). Renou, M. *et al.* (1981) identified (*Z*)-11-hexadecen-1-al (Z11 : 16AL) as the major component of the pheromone. They demonstrated a graded discrimination of Z11 : 16AL and five analogs at three concentrations by monitoring the progressively higher-order behaviors. At the lowest concentrations tested, four of the five analogs elicited the lowest order behavior, but only the pheromone elicited the complete behavioral hierarchy. Only at concentrations 10,000 times higher did the analogs elicit behaviors above the lowest order. In the field trapping studies, indicative of yet more stringent behaviors, only traps baited with the pheromone at still higher concentrations than in the laboratory assays actually captured any males. Such interactions in the response to quality and quantity complicate any study of the linkage between the behavioral response and peripheral sensation of pheromone, and many additional studies are required to explain discriminative processes.

6.2.2 Genetic alteration of the neurophysiological and behavioral response to pheromone

Genetic manipulation has been used often as a tool for identifying receptor proteins in unicellular organisms, but insect neuroethological genetics is still in its infancy. It is difficult to separate by behavior genetic alteration of the central reflex mechanisms from alteration of the peripheral receptor cells. Although olfactory behavior can be altered genetically (Kikuchi, T. 1973; Norris, D. 1976; Rodrigues, V. and Siddiqi, O. 1978; Fuyama, Y. 1978), the altered behavior alone is not *prima facie* evidence for a genetic alteration of the receptor cell and thus of a receptor protein. Consequently, this subsection is rather brief and speculative.

The best evidence for genetic alteration of primary olfactory receptor cells is from studies by Priesner, E. (1979) of F_1 hybrids of *Zeiraphera diniana* (Gn.). This species has two forms (or races); one, the cembran pine form, exhibiting an EAG maximally responsive to (*E*)-11-tetradecen-1-ol acetate (E11 : 14AC) and much less sensitive to (*E*)-9-dodecen-1-ol acetate (E9 : 12AC). The other race, the larch form, exhibits an EAG maximally responsive to E9 : 12AC and less to E11 : 14AC. The F_1 hybrid exhibits an EAG equally sensitive to both compounds and the single cells also exhibit the hybrid pattern. Priesner suggested that the receptor sites for the parental pheromones were combined on the same sensory cell. Combined genetical and electrophysiological studies of races of both this species and *Ostrinia nubilalis* (Klun, J. and Maini, S. 1979) could provide further information about peripheral receptors and the central control of olfactory discrimination.

It may be more difficult to reconcile electrophysiological evidence with behavior if *Z. diniana* or *O. nubilalis* are proven to have variously responding receptor cell families as in *Trichoplusia ni*, *Argyrotaenia velutinana*, and *Lymantria dispar*.

6.2.3 Habituation, adaptation, inhibition, synergism and other modifications of pheromonal responses

Several interrelated exogenous and endogenous factors have been reported to affect behavioral responses to pheromone. The proper methods of study, as well as the terms used to describe the behavioral modifications, are poorly defined. Perhaps after the term attraction the most oft misused terms in pheromone neurobiology and behavior are adaptation, inhibition, and synergism. Thompson, R. and Spencer, W. (1966) make no distinction between the terms habituation and adaptation, while Eisenstein, E. (1972) defines habituation as "... the progressive decrease in magnitude and/or probability of a response resulting from its repeated elicitation *by an intermittant stimulus*" [our italics]. Thompson, R. and Spencer, W. (1966) were concerned predominantly with behavioral responses, and Eisenstein with learning and memory in an isolated ganglion. Nine criteria defining habituation are given in Eisenstein, quoting from Kandel, E. and Spencer, W. (1968), but little experimental effort has been devoted to applying these definitions either to the behavioral response or to the response of the peripheral receptor cells.

We prefer to separate these two terms by specifying, for example, that behavioral habituation to a pheromone, its analogs, isomers, etc., is never clearly indicative of either central or peripheral diminution of response, just as behavior is never clearly indicative of peripheral sensation. Receptor adaptation obviously would be the preferred usage to define, for example, a diminished EAG response to a large pheromonal stimulus (Kaissling, K. 1977a). Behavior alone cannot distinguish between central habituation and peripheral adaptation. It thus appears to us that either term used from behavioral criteria alone becomes a portmanteau term for a final result.

Bartell, R. (1977) has discussed most of the extant and relevant information on habituation. We also note that although the EAG shows adaptive effects, the individual cells of *Bombyx mori* apparently adapt little if at all to repeated small stimuli (Kaissling, K. and Priesner, E. 1970). Some of the HSA cells of *T. ni* exhibited a slow but progressive and profound adaptation to low constant stimulation with Z7 : 12AC, other cells did not adapt within 30 min (Mayer and Mankin, unpublished data). In any circumstance the presence of families of pheromone-sensitive cells on the antenna complicates interpretation of receptor-cell adaptation.

The role of hormones in modifying or releasing pheromonal as well as other behaviors has been considered in some detail by Truman, J. and Riddiford, L. (1974, 1977). They group the effects on behaviors mediated by hormones into "modifier" or "releaser" effects, the releaser effects not necessarily being mediated through sensor-mediated mechanisms. Conceptually it is a bit difficult to separate the releaser effects of hormones from those effects produced by inhibitory or excitatory neurotransmitters. The described modifiers appear mostly to affect the less obvious sexual behaviors induced by pheromones in Orthoptera and Diptera.

Two hormonal releaser effects have been described for sexual behavior: one is the "calling" behavior of females, discussed in section 3.1, and another is maturation induced by the eclosion hormone. The concept of a host volatile, (*E*)-2-hexenal, earlier reported by Riddiford, L. (1967) to release calling behavior, has been questioned from a parallel study (Cardé, R. and Webster, R. 1980). The initiation of behavioral responses to pheromone in *Antheraea pernyi* is reported by Truman, J. and Riddiford, L. (1974) to be released by eclosion pheromone (Truman, J. *et al.*, 1981). The purported evidence consists of observations that uneclosed males, peeled from the pupal case, will not mate with virgin females even though the peripheral antennal receptor cells can respond to the pheromone (monitored by the EAG). They mate only after they have aged further until the normal time of emergence and have undergone an emergence sequence.

6.2.4 Behavioral thresholds

Apparently every component of the hierarchy of events that result in pheromone-induced sexual behavior requires a specific minimum level of pheromone—a threshold level. The observations of Schwinck, I. (1956) on *B. mori*, and Renou, M. *et al.* (1981) in general support this concept. Shorey, H. (1977) described a similar sequence of behavioral events in *Trichoplusia ni*. Schwinck's study showed that the earlier phases in the behavior require lower pheromone concentrations than the later phases. It should be noted that habituation and environmental factors also can affect the threshold.

There are only a few experiments where the threshold has been ascertained under conditions that allow an estimate of the pheromone dose in physiologically significant units of molecules per cm^3 (Table 4). It is interesting that the criterion behavior was elicited at such low levels in all instances by a single chemical when all the species tested, except *Lymantria dispar*, are known to have two or more chemicals comprising the complete pheromone bouquet. We thus might surmise that the pheromone component released in the greatest quantity is responsible primarily for "activation" of the insect. More specialized behaviors may be mediated by the addition of other chemicals to the bouquet as demonstrated by Baker, T. *et al.* (1976).

6.2.5 Quantifying the behavioral power functions

The most common method of quantifying the behavioral response to pheromone is to perform a bioassay and then to calculate a threshold by probit analysis (Mankin, R. *et al.*, 1980a). But this method obtains less information about the response than a

*Table 4: Behavioral thresholds of insects to major components of their pheromones**

Insect, pheromone component, temperature	Threshold concentration (10^3 molecules per cm^3)	References
Bombyx mori		
(*E,Z*)-10,12-hexadecadien-ol		Kaissling, K. and Priesner, E. (1970)
17°	20	
21°	10	
Trichoplusia ni		
(*Z*)-7-dodecen-1-ol acetate		Sower, L. *et al.* (1971)
24°	8	
Plodia interpunctella		
(*Z,E*)-9,12-tetradecadien-1-ol acetate		Mankin, R. *et al.* (1980b)
23°	1,340	
34°	16.5	
Trogoderma glabrum		
(−)-14-methyl-(*Z*)-8-hexadecenal		Shapas, T. and Burkholder, W. (1978)
27°	2,300	
Lymantria dispar		
cis-7,8-epoxy-2-methyloctadecane		Aylor, D. *et al.* (1976)
25°	115	

* From Mankin, R. *et al.* (1980). Other thresholds are reported in Kaissling, K. (1971). The behavioral response criterion was upwind anemotaxis for *Plodia interpunctella* and orthokinesis for the other insects.

different method derived from electrophysiological and vertebrate psychophysical studies. The second, more informative method is to calculate the power function of best fit to the bioassay data rather than the best fitting probit function. The bioassay power function has the form

$$R = b_1(C+b_2)^b, \qquad \text{(XX)}$$

where R is an appropriate measure of response, C is the pheromone concentration, and b, b_1 and b_2 are regression constants. Bioassay power functions are considered briefly in section 4.7 (see eqns XVIII and XIX) and in more detail in Mankin, R. and Mayer, M. (1983a,b).

The utility of the power function analysis stems from two considerations. First, the exponent b in eqn XX is independent of the stimulus and response units. Theoretically, the exponent for any bioassay power function measuring a response that correlates with the perceived intensity of the olfactory stimulus should be less than or equal to the exponent for the power function of perceived intensity. Thus, measurement of the exponent of the bioassay function enables the behavioral physiologist to estimate a lower limit for the exponent of the perception function when the perception function cannot be measured directly. It also follows that bioassays measuring different kinds of response to a pheromonal stimulus, e.g., wing fanning or anemotaxis, can be compared directly through the exponents of their power functions.

The second consideration enhancing the utility of the bioassay power function analysis is that the negative of the regression constant, b_2 in eqn XX, is a separate measure of the behavioral threshold. Two examples of this are given in eqns XVIII and XIX, where the anemotactic threshold of *Plodia interpunctella* to its sex pheromone is calculated to be about 1×10^{-4} μg dose (see also Table 4).

The power function analysis puts into proper perspective the threshold concept. It is apparent that, although knowledge of the threshold provides considerable information about the behavioral response, the threshold alone is only a single point on a continuum of behavioral response. As behavioral analyses become more sophisticated, it will become more important to consider the continuum rather than just the threshold.

7 CONCLUDING REMARKS

Investigations of the neural responses of insects to pheromones and other odorants began just 25 years ago, the discipline progressing somewhat sporadically, reflecting the small number of interested

groups and isolated nature of the studies. The present generally accepted concepts of the workings of the peripheral olfactory system are primarily the result of a small number of excellent investigations on a unique species, *Bombyx mori*. We discuss below the areas of investigation where detailed knowledge is available, where it is not, and attempt to identify what we believe are promising areas for study in the future.

7.1 Areas where knowledge is substantial

There are a variety of facts about the olfactory system of insects that most investigators accept virtually without question. Into this category can be placed the generalized descriptive morphology of receptor cells, the multiporous outer cuticular covering of the dendrites, and the connection of their axons to glomeruli in the deutocerebrum. Also accepted is that the olfactory information is transmitted to the brain as a series of action potentials that code quality and quantity. Furthermore, the physical and chemical principles that govern pheromone dispersion in the air and its diffusion along the receptor surfaces, at first misconceived, are now clearly established. Many of the investigations establishing these principles, however, are reported predominantly in chemical engineering journals and therefore are not well known to neurobiologists. The limits of morphological diversity of sensory structures appear reasonably well defined. Behavioral thresholds can probably be estimated within an order of magnitude or so by measuring the total surface area of the pheromone-sensitive sensilla.

It is also known that the interaction of a pheromone molecule with a sensory cell dendrite results in action potentials. A further safe assumption for *Bombyx mori*, and probably other species as well, is that single molecules of its major pheromone component will elicit one or more action potentials. A complicating factor to interpretation of CNS and behavioral responses to pheromone is that the qualitatively and quantitatively differing response groups of cells which occur are morphologically distinguishable only at high magnification ($> \times 20{,}000$). A caveat important enough to recapitulate is that the *lack of a behavioral response is not evidence of non-sensation*. The correlation of structure–activity relationships based on behavior bears a tenuous relationship to transduction. Genetic studies using behavioral responses as the basis for conclusions relative to transduction and "active sites" of proteinaceous receptors are also questionable. Obviously, this caveat alone is sufficient to question such investigations into receptor sites, etc., but a further confounding factor in interpreting behavioral and EAG results are the multiple groups of cell types that respond to pheromone. Admittedly, often the results of such experiments are most intriguing; regrettably, they have no firm foundation.

7.2 Areas where knowledge is lacking

So much is not known about pheromone neurobiology, and so many misconceptions have developed because of this lack of knowledge, that it is difficult to confine this discussion to a reasonable space. The following topics are chosen as much for their relationship to areas where knowledge is substantial as to their potential to provide new concepts.

The most basic uncertainty is the means by which a pheromone molecule arriving at the receptor cell dendrite is able to initiate the characteristic potential changes across the membrane. Certainly, the overwhelmingly accepted mechanism is one involving a weak binding of the molecule to a membrane-bound protein. No receptor protein as yet has been isolated, and the presence of multiple receptor cell groups on the antenna suggests that this biochemical task may be more difficult than earlier envisioned. Likewise, the very small size of the dendrite within the sensillum eliminates any possibility of measuring the intracellular potentials developed in transduction.

The means by which pheromones and blends of pheromone components are discriminated by peripheral receptor cell groups can be assessed more easily now, but the process of behavioral release by complicated interactions within the CNS is wholly unknown. Even the descending fibers to the suboesophageal ganglion have not been located. It appears safe to surmise that threshold-level discrimination of pheromone blends by an individual cell is unlikely. This would require essentially simultaneous interactions of the different molecules in the blend with a single receptor site.

Near the threshold such an interaction is statistically very improbable.

It is not known as yet how many neurophysiologically similar groups of receptor cells can be identified on the antenna of any species of insect, nor is there a completely reliable system as yet for distinguishing among groups. The variability of responses within a group, which is an important parameter for distinguishing among groups, may overlap the variability of responses among groups. This further complicates the task of classification. The presumably slight intrusive effect of the recording technique itself has not been studied. How do we, in fact, assure ourselves that at least some of the observed effect is not due to instrumentation?

A final area of uncertainty concerns the effect hormones may have directly or indirectly on the behavior of responding and emitting sexes. Such effects, if they exist, are expected to be difficult to measure and interpret.

7.3 Promising areas for future study

The preceding section, identifying areas where knowledge is lacking, reveals some important but difficult areas for study that require sophisticated instrumentation, specialized knowledge, or both. In this section we try to identify also some areas where knowledge is lacking but which require less sophisticated equipment or which have an applied use based on what is known.

The brain can be identified as a most promising area for study for several reasons; the first is that few histomorphological studies have been directed toward tracing known olfactory fibers that lead from the deutocerebrum. As an example suggested in the text, there may be a few pheromonally activated interneurons that project directly to the suboesophageal ganglion. The possibility also exists that inhibitory interneurons project into the deutocerebrum. Such histomorphological studies are essential for neurophysiology. Differences among a variety of species, if found, could provide working models for examining and isolating particular pheromone-induced behaviors. Such findings would yield requisite foundational data for neurobiological investigations into the neural origins of olfactory behavior. Comparative studies would also provide an assessment of the range of differences neurobiologists might expect to find between the brains of various species, families and orders of insects.

Detailed scrutiny of particular areas of the brain is needed, particularly those within the deutocerebrum and especially the macroglomerulus. Only a few generalizations as yet can be made about the macroglomerulus, but already this structure holds extraordinary implications. Likewise, countable numbers of output interneurons projecting into the mushroom bodies from the macroglomerulus conceivably can provide keys to discrimination of pheromones.

The discussions within this chapter perforce have been centered on laboratory studies and are what could be termed basic science. Behavior and the neurobiological basis of behavior must ultimately be examined in the natural habitat of the animal. For obvious reasons some behavioral and neurobiological studies cannot be performed outside the laboratory, but many can; at least enough to ensure that the laboratory tests are indicative of real effects. Although the linkage of pheromone neurobiology into integrated pest management may appear a bit futuristic, some of the results of these neurobiological studies can be linked now to applied uses. Perhaps the most important immediate linkage can be exemplified by combining the morphometry of antennal sensilla with behavior and receptor cell threshold analyses into a universal model to calculate the active space of sex pheromones. Also, investigations of receptor cell discrimination may lead to the discovery of chemicals that interfere with pheromone-induced communication. We therefore conclude that there are many areas of pheromone neurobiology that, with the proper knowledge and ingenuity, can be used in applied entomology.

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