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Sensitivity of the Contact Chemoreceptors of the Blowfly to Vapors

(common chemical sense/gustation/olfaction/sensory coding/aversive behavior)

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ABSTRACT **Contact chemoreceptors on the mouthparts and legs of the blowfly *Phormia regina* that normally respond to aqueous solutions of sapid substances also respond to compounds in the gaseous state. Effective vapors include organic and inorganic acids and various unrelated nonpolar compounds. In general, the acids stimulate the salt receptor. Some nonpolar compounds stimulate the salt receptor while others inhibit it. Others stimulate the water, sugar, or "fifth" receptor. Differential action cannot be attributed to pH or solubility. Not all compounds that are irritating to mammalian mucous membranes or amphibian skin stimulate the contact chemoreceptors of the fly. Sensitivity to these vapors is a phenomenon analogous to the common chemical sense of vertebrates.**

Before the advent of electrophysiological techniques, the identity and distribution of chemoreceptors of insects was established by a combination of topical application and ablation with behavioral observation. Although these methods established the preeminence of antennae as the sites of olfaction and of the mouthparts and legs as sites of gustation, there continued to be reports of residual sensitivity to vapors after removal of all known olfactory receptors. In particular, McIndoo (1, 2) consistently reported a general body sensitivity to concentrated vapors of many compounds. He concluded, erroneously, that the widely distributed campaniform sensilla were "olfactory pores." His conclusions and those of others who argued for a more or less generalized olfactory sense fell into disrepute and were eventually forgotten. Nonetheless, the idea that insects might possess a common chemical sensitivity to vapors analogous to the skin sensitivity of amphibians and to the sensitivity of mucous membranes of man continued to invite discussion (3). Electrophysiological analyses have now provided evidence for sensitivity in insects that is compatible with the concept of a common chemical sense.

MATERIALS AND METHODS

The labellar chemosensory hairs of the blowfly *Phormia regina* are primarily gustatory organs. A typical hair is equipped with five bipolar neurons of which one is a mechanoreceptor. The dendrites of the others terminate in an apical pore in the hair (Figs. 1 and 2) where they normally come into contact with liquids when the hair touches the substrate. One dendrite is preferentially sensitive to certain carbohydrates and amino acids, one is sensitive to water, and the remaining two are sensitive to salts and miscellaneous compounds.

The response characteristics of these receptors are best studied by the side-wall technique of recording originally perfected by Morita and Yamashita (4). The isolated head is impaled on a glass micropipette containing 0.1 M NaCl that serves as a salt bridge to a Ag/AgCl wire. This is the reference electrode. A similar pipette and wire serving as a recording electrode makes contact with the dendrites through a crack in the side of the hair, thus leaving the apical pore available to any stimulus. All recording is extracellular.

The presence or absence, as well as the nature, of the elec-

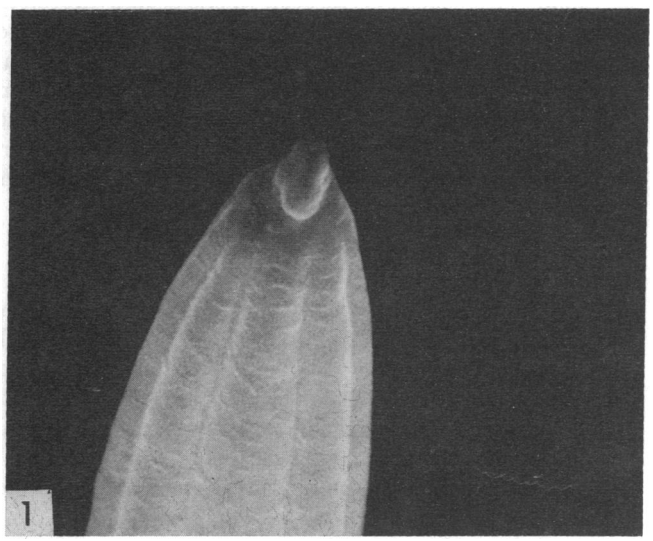

FIG. 1. Largest type of labellar hair showing exudate at tip. (×12,000) (Photographed with the scanning electron microscope at Florida State University, V. G. Dethier and R. Parker.)

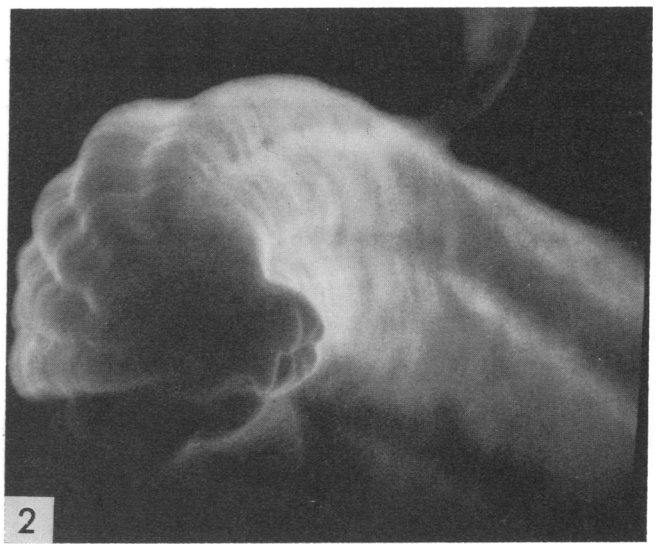

FIG. 2. Recurved type A tarsal hair showing dimpled tip free of exudate (×20,000) (Photographed with the scanning electron microscope at Florida State University, V. G. Dethier and R. Parker.)

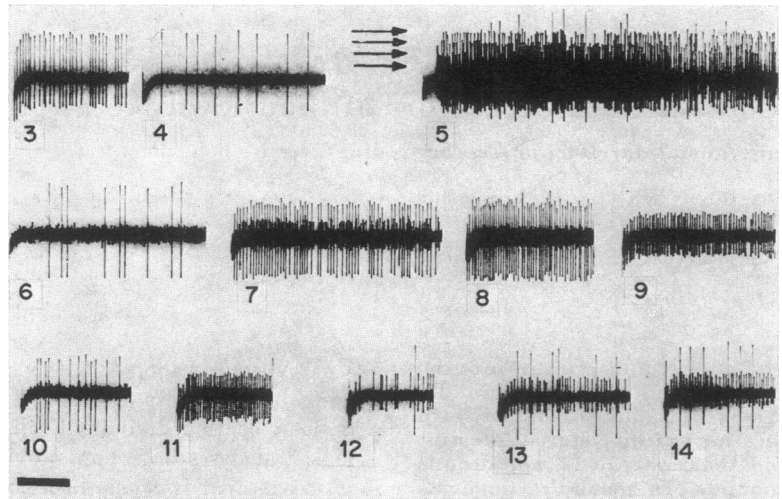

FIG. 3. Response of largest labellar hair no. 1 to 0.1 M sodium chloride. *Horizontal bar* in Figs. 3–14 equals 0.2 sec.
FIG. 4. Response to the vapor of formic acid.
FIG. 5. Response to a solution of 2.66 M formic acid. Four cells responded. Base line silent before stimulation.
FIG. 6. Response of largest hair no. 5 to 0.1 M NaCl.
FIGS. 7,8,9. Responses to 6 M NaCl, vapor of formic acid, and vapor of citronellal, respectively. Baseline silent before stimulation in Figs. 6–9.
FIG. 10. Active baseline.
FIG. 11. Response to the vapor of benzene.
FIG. 12. Baseline for largest hair no. 2.
FIGS. 13,14. Responses to a solution of 0.1 M NaCl and to the vapor of formic acid, respectively.

trical activity of an unstimulated hair depends upon the circumstances of contact with the electrode. With the most gentle of contacts, there is practically no electrical activity recorded from any dendrite. The baseline may be absolutely silent; however, the more usual state is one of infrequent action potentials from one or more cells. The more traumatic the contact, the more active are the neurons. Regulation of the amount of activity in the neurons through adjustment of the recording electrode presents opportunities to assess the inhibitory as well as the excitatory effect of stimulating compounds.

TABLE 1. *Types of responses elicited from labellar gustatory hairs by miscellaneous vapors*

Compound	Classical salt receptor	Water receptor	Sugar receptor	Fifth receptor
HCl (low conc.)	+	0	0	0
(high conc.)	+	+	+	+
HNO_3 (low conc.)	+	0	0	0
(high conc.)	+	+	+	+
Acetic acid	+	0	0	0
Formic acid				
(low conc.)	+	0	0	0
(high conc.)	+	+	+	+
Valeric acid	+	+	+	+
Citronellal	−	0	?	?
Citral	−	+	?	?
Limonene	−	0	?	?
Isobutyraldehyde	Off effect*	0	0	0
Napthaquinone	0	+	0	0
Benzaldehyde	−	+	0	+
Benzene	−	?	?	?
Xylene	0	0	0	0

* Salt receptor generates action potentials only when stimulus is removed.

Compounds to be tested were placed in a glass pipette (tip diameter about 6 μm) that was then moved slowly toward the tip of the hair. Neural activity was monitored continuously as the pipette approached to within 200 μm of the hair and was then withdrawn. After each stimulation, the neurons were allowed to return to their basal rate of activity. Periodically they were stimulated with water, 0.1 M NaCl, and 0.5 M sucrose to ascertain whether or not they were still responding normally to physiologically adequate stimuli.

Test stimuli included the following compounds applied as vapors: formic acid, acetic acid, valeric acid, hydrochloric acid, nitric acid, ammonium hydroxide, allyl-isothiocyanate, ethanol, isobutanol, pentanol, octanol, methanal, isobutanal, heptanal, octanal, citral, limonene, citronellal, linalool, menthol, oil of peppermint, hydroquinone, toluquinone, benzaldehyde, xylene, benzene, and carbon dioxide. Liver extract, brain–heart extract, and several natural foods (e.g., decaying meat) were also tested. Concentrations were not regulated.

RESULTS

The results of stimulation by vapors are summarized in Table 1 and illustrated in Figs. 3 to 21. No responses were obtained to vapors normally associated with food, i.e., carbon dioxide, liver extract, brain–heart extract, and meat. Responses occurred only upon stimulation by vapors that would be characterized as nonphysiological; but the presence, absence, and nature of response varied from one compound to the next.

All of the acids caused an increase in the rate of firing of the classical salt cell (Figs. 4, 8, 14, and 16). As the concentration of vapor increased, the rate of firing increased. At

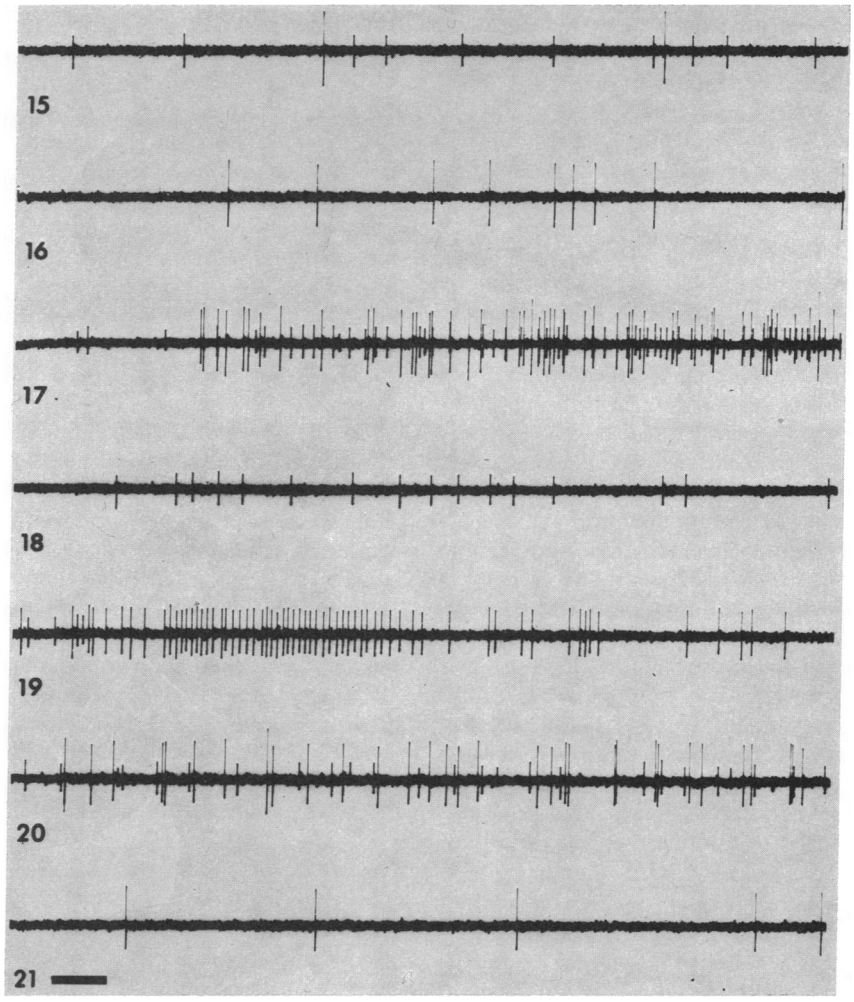

FIG. 15. Baseline activity in largest labellar hair no. 2. *Horizontal bar* in Figs. 15–21 equals 0.1 sec.
FIG. 16. Response of hair no. 2 to vapor of formic acid.
FIGS. 17–21. Responses to vapors of valeric acid, citral, limonene, benzene, and xylene, respectively.

high concentrations, the other three chemoreceptive cells also responded (Fig. 17). Upon removal of the vapor, the neural activity continued for as long as 10 sec when the stimulating concentration had been high. All cells subsequently returned to their basal rate of activity and responded normally to water, sugar, and salt. The classical salt cell was the most sensitive.

None of the cells responded to ammonium hydroxide or allyl-isothiocyanate, both of which are extremely irritating to the mucous membranes of man.

The possibility that the cells were responding only to extremes of hydrogen ion concentration was ruled out by the results obtained with various nonpolar compounds. In general the nonpolar compounds inhibited the classical salt cell (or failed to influence it in any way) and excited the water, sugar, or fifth cell (Figs. 9, 11, and 18–21).

DISCUSSION

It is clear that concentrated vapors of several unrelated compounds are capable of stimulating the gustatory receptors of the blowfly. Obviously, these compounds pass from the vapor state into solution at the dendritic terminal. Exactly what fluid is involved remains a mystery. A sizeable exudate is frequently observed on the tips of chemosensory hairs (Fig. 1). Stürckow (5) views this exudate as a normal phenomenon essential for the process of transduction. She has advanced the working hypothesis that the substance is an acceptor–donor type, the molecules of which develop a current flow at the moment of absorption of stimulus molecules. Examination of a great number of labellar and tarsal hairs with a scanning electronmicroscope revealed that the drops in question seldom occur on tarsal hairs and are of sometime occurrence on labellar hairs irrespective of whether the hairs were prepared by gold plating or examined fresh. Figs. 1 and 2 show examples of hairs with and without exudate.

It is inconceivable that the dendrites not be bathed in fluid. Whether this is a monolayer or a copious exudate may be unimportant. In any case, stimuli must pass into or through a fluid enroute to the dendritic membrane. Vapors that do stimulate presumably enter into solution or form a two-phase liquid system with the exudate, which then brings them into apposition with the neural membrane. The fact that polar and nonpolar compounds and compounds of different solubilities do stimulate and that different individual neurons respond differently to any given compound suggests a passive nonspecific roll for the exudate.

The data in Table 1 indicate that the effectiveness or ineffectiveness of a compound is not simply a function of its

solubility in the fluid of the receptors. It is unlikely that ineffectiveness derives from an inability to enter into solution because compounds with similar solubility characteristics may or may not stimulate (see xylene and benzene, Figs. 20 and 21, and formic acid and ammonium hydroxide). It is equally clear that the effects observed cannot be attributed solely to pH; that is, response is not a matter of abusing the system with nonphysiological hydrogen ion concentrations. Finally, not all compounds that affect the common chemical sense of men and frogs stimulate the labellar hairs. In addition of ammonium hydroxide, allyl-isothiocyanate may be cited as an example of ineffective "irritants."

Considered together the foregoing facts indicate that the sensitivity of labellar receptors to vapors is markedly specific. When the patterns of response are analyzed, it is seen that the different compounds elicit characteristic kinds of responses. Citronellal, for example, inhibits the salt receptor and stimulates either the sugar or fifth receptor. Limonene acts in a similar fashion, whereas citral stimulates the water receptor. Isobutyraldehyde does not stimulate any receptor, but when it is removed the salt receptor responds with a burst of activity. Xylene is generally ineffective, but benzene stimulates the water receptor and one other receptor.

All of the hairs tested were the largest type (6). Not all responded alike to various vapors. This is consistent with the fact that the hairs vary with respect to sensitivity. There are also indications that with side-wall recording the sensitivity of a receptor to chemical stimulation is influenced by the degree of stimulation that is introduced by the recording electrode itself.

Certain aspects of these responses are reminiscent of the electrical responses of olfactory receptors in which the basal level of activity in the absence of stimulation may be raised by some stimuli and decreased by others (7–10). In general, the vapors of nonpolar compounds tend to inhibit activity of the salt cell while the polar compounds tend to stimulate this cell. The nonpolar compounds vary with respect to their effectiveness on the nonsalt receptors. Some stimulate the water cell; some stimulate the sugar or fifth cell; others inhibit. Still others that cause no demonstrable excitation or inhibition do cause a marked off-effect in one or more receptors. The occurrence of off-effects in one or more cells indicates that a given compound may affect receptors and affect them differentially, even though no action potentials are generated at the time of contact.

Off-effect, or rebound, in gustatory receptors of insects was first reported by Morita and Yamashita (4), who showed that the labellar hairs of the fly *Calliphora* generated action potentials after stimulation with $CaCl_2$. McCutchan (11) reported similar effects in the tarsal chemosensory hairs of *Phormia*. More recently, Goldrich (12) has shown that stimulation of some labellar hairs of *Phormia* with water is followed by a sharp burst of activity from the salt receptor. In the case of $CaCl_2$ the salt receptor was hyperpolarized while the solution was touching the dendrite and depolarized when the solution was removed. Similar situations may prevail when vapors cause inhibition and rebound.

The behavior of gustatory receptors with respect to concentrated vapors greatly resembles the behavior of olfactory receptors toward odorous compounds. The resemblance raises the possibility that the manner in which olfactory receptors are excited and inhibited differentially is not specific to those systems but instead reflects a general characteristic of neurons. Comparable phenomena appear in odd places. Arvanitaki *et al.* (13) have shown, for example, that certain neurons in the central nervous system of *Aplysia* react selectively to "odorous" molecules by depolarizing or hyperpolarizing and generating spike discharges of specific frequency-time relationships.

The initiation by vapors of action potentials in the contact chemoreceptors of the fly indicates that information is transmitted to the central nervous system. The fly does act behaviorally on some of this information. When all known olfactory receptors are extirpated, an intact fly responds to vapors of formic acid, citronellal, etc., by aversive movements of the proboscis (14, 15). When vapors are brought close to the tarsi, tethered flies retract the legs. Although the small size of tarsal chemosensitive hairs prevents electrophysiological recording from any but the largest, records from D hairs reveal a sensitivity to vapors comparable to that characterizing labellar hairs. These findings suggest that all the contact chemoreceptors of *Phormia* may behave similarly, and offer an explanation of the function of contact chemoreceptors that are located on areas of the body where contact with solutions is normally unlikely. Wolbarsht and Dethier (16) showed, for example, that *Phormia* possesses on the costa of each wing a row of short hairs that generate action potentials in response to the application of sodium chloride. No function was proposed at the time. If these hairs resemble labellar hairs in their sensitivity to vapors, they might, in common with other contact chemoreceptive hairs subserve the function of a common chemical sense. Since flies are not likely to encounter concentrated formic acid, etc. in nature, the adaptive value of this common chemical sense remains an unanswered question.

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