

# Candidate Codes in the Gustatory System of Caterpillars

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**ABSTRACT** Larvae of tobacco hornworms offer unique opportunities to relate the electrophysiological output of identified chemosensory neurons to specific behavioral responses. Larvae can discriminate among three preferred plants with only eight functioning gustatory receptors. They can be induced to prefer any one of the plants, and these preferences can be reversed. All eight neurons respond to each plant sap. Two fire too infrequently to permit detailed analysis. Analyses of the remaining six show that all electrophysiological responses consist of phasic and tonic components. Only the "salt best" cell fires during the phasic period. Temporal analysis of the spike train during this period shows that tomato and tobacco could be distinguished from Jerusalem cherry but not from each other by a rate code. Measurements of behavioral response times together with the nonspecificity of this with respect to food plants, unacceptable plants, and sodium chloride eliminate a phasic period rate code as a probable mechanism for complex discrimination. Events occurring in the tonic period, when all cells are firing, suggest a major role for this period. Analyses of variance in the interval frequencies of the large and medium spikes suggest that a variance code could allow discrimination among the three plants as long as both cells were firing at the same time. Evidence has been found for temporal patterning in the tonic response of the "salt best" cell to Jerusalem cherry but is absent elsewhere. The most likely basis for coding the difference between each of the three plants is across-fiber patterning in which the relative rates of firing and the variances of all the sensory neurons in the tonic phase are critical.

Although the choice of plants by phytophagous insects involves integration of information provided by many sense organs (visual, tactile, olfactory, and gustatory), it is well documented that the gustatory system plays a leading role in feeding and that a high level of discrimination by caterpillars is still possible when all chemosensory input except that from gustatory receptors is blocked (Hanson and Dethier, 1973). Furthermore, it has been postulated that the preferential selection and acceptance of food is based upon a capacity to discriminate plants qualitatively (Dethier, 1976*a* and *b*, 1977, 1978). In other words, plants of various degrees of acceptability are not discriminated because

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they elicit different intensities of a single, albeit rather complex, stimulus quality, but because they elicit qualitatively different perceptions.

This hypothesis is derived from the following facts: insects can distinguish one acceptable plant from another by chemosensory means as shown by behavioral choice tests and learned preferences (Dethier, 1937, 1941; Jermy et al., 1968; Städler and Hanson, 1976, 1978); different acceptable plants are chemically qualitatively different (Dethier, 1941; Rodman and Chew, 1980); there is sufficient specificity among receptors such that different plants stimulate different neural units (cf. Schoonhoven, 1967; Wieczorek, 1976); gustatory and olfactory electrophysiological responses to a plant are multineuronal in all cases, and different plants evoke different neural patterns (Dethier and Kuch, 1971; Dethier, 1973); there is no single electrophysiological response pattern signaling acceptability, nor is there a single pattern signaling unacceptability (Dethier, 1973). If this hypothesis is correct, there must be sensory codes not only for gustatory intensity but for quality as well.

The existence of such neural codes and their perceptual reality can be demonstrated only if specific electrophysiological events in sensory fibers can be detected and correlated unequivocally with behavioral discrimination. The customary method for evaluating the ability of insects to discriminate among plants employs a two- or many-choice paradigm in which the measured variable is the weight of leaf consumed in a given time interval. This type of test does not, however, enable the experimenter to distinguish between preferences that are based on quantitative differences in taste and those based on qualitative differences. A closer approximation to proof of the qualitative nature of a gustatory discrimination can be achieved by showing that preferences can be learned and that the learning can be reversed. Even this demonstration does not provide an absolute criterion because theoretically an insect could learn to prefer a given intensity of stimulus and subsequently be trained to prefer a different intensity; however, the test is the most rigorous currently available.

Larvae of the tobacco hornworm (*Manduca sexta*) can be induced to prefer a particular plant by feeding them exclusively on that plant for a period of 72 h or more (Jermy et al., 1968). The slight preferences of "naive" animals for Jerusalem cherry (*Solanum*) can be reversed by a feeding experience on tomato (*Lycopersicon*), and the preference for Jerusalem cherry can be greatly augmented by a prior feeding on that plant.

It has been demonstrated that discrimination and induction are possible when all chemoreceptors except the maxillary sensilla styloconica and the epipharyngeal sensilla are ablated (Hanson and Dethier, 1973). These experiments also militate against the involvement of some internal receptors or non-neural systems as, for example, enzyme induction (cf. Schoonhoven, 1969). The contribution of the epipharyngeal receptors appears to be concerned principally with swallowing. They are less discriminating than the maxillary receptors and are considered to be a second checkpoint for feeding deterrents (de Boer et al., 1977).

From these considerations it appears that sensory information from the two

maxillary sensilla, which together contain a total of eight primary receptor neurons, is sufficient to enable the caterpillar to make a qualitative assessment of its foodplants. The electrophysiological study reported here sought to discover relevant characteristics of the sensory neural message that make this postulated qualitative discrimination possible.

#### METHODS

Electrophysiological tests were conducted on fourth and fifth instar larvae of the tobacco hornworm, *Manduca sexta* (Johanssen). The larvae, supplied by the United States Department of Agriculture from a laboratory-reared strain, were maintained on a synthetic diet (Yamamoto, 1969) before testing. Leaf saps were expressed from fresh leaves of tomato (*Lycopersicon esculentum* Mill.) tobacco (*Nicotiana tabacum* L.), and Jerusalem cherry (*Solanum pseudocapsicum* L.), drawn into glass capillaries, and placed on the tip of a gustatory sensillum. The entire process was accomplished in 20–30 s.

Although there is no assurance that rapid enzymatic changes had not already begun, the elapsed time is of the same order as would occur in normal feeding sequences on acceptable plants. As the caterpillar chews rhythmically along the edge of a leaf, it encounters fresh sap and sap extruded during earlier sweeps. It behaves the same toward each sap.

The gustatory organs of each maxilla are two sensilla styloconica, one medial, the other lateral. Each sensillum consists of four bipolar sensory neurons the axons of which extend directly into the suboesophageal ganglion. Separate recordings were made from each sensillum by placing the pipette containing the leaf sap on the tip. The pipette thus acted as a vehicle for the delivery of stimuli and as a recording electrode. An indifferent electrode was inserted into the foramen magnum of the amputated head. Action potentials were amplified by a Bioelectric neutralized input capacitor amplifier type NFI (Bioelectric Instruments Inc., Hastings-on-Hudson, N. Y.). The input leakage current was  $3 \times 10^{-13}$ – $10^{-12}$  A.

Medial and lateral sensilla were tested on each of 10 caterpillars with each of three leaf saps. Each test of a sap consisted of five 10-s stimulations separated by 10-min intervals. Saps were replenished at each interval. Each series of five stimulations was repeated three times in different orders of presentation. The total number of stimulations, counting both sensilla, was 900. In addition, a total of 400 stimulations was made with four concentrations of tomato leaf sap (25, 50, 75, and 100%). 400 more stimulations were made with similar concentrations of Jerusalem cherry leaf sap and will be reported in a subsequent communication. A few preliminary experiments were also carried out with saps of carrot and canna, unacceptable plants, and *Petunia*, an acceptable but toxic plant.

Since it was not possible to record from each receptor cell individually, each record included activity from one to four cells (in each sensillum), depending upon the nature of the stimulus. The fourth neuron, the one giving the smallest spike, fired so infrequently that it was not included in the analysis. Neurons were identified by the amplitudes of their action potentials.

In the phasic period, firing rates (reciprocal of interspike interval in the first 100–150 ms) were measured for each kind of stimulus and compared. The significance of differences among comparisons was tested by analysis of variance using Duncan's Multiple Range Test (Steel and Torie, 1960).

In the tonic phase, spike frequencies and interval distributions during the third second were measured by hand and the standard statistical quantities were computed; mean interval ( $\mu$ ), standard deviation ( $\sigma$ ), interval variance ( $\sigma^2$ ), and coefficient of

variation ( $\delta/\mu$ ). These quantities were calculated for the third, fifth, and ninth seconds of response. Since the data obtained from each of these time samples were nearly identical, only one sample (the third second) is discussed in detail. Significance was tested by the Kolmogorov-Smirnov test (Siegel, 1956).

To test for the possible occurrence of temporal patterns in spike trains during the tonic phase, statistical measures for order dependence in the firing pattern of the large spike were made by computer. First- to fifth-order spike interval distributions were measured from one sample record of the response of a single individual sensillum of tomato, tobacco, and Jerusalem cherry. The sample was the first 10 s of response from time 200 ms after the onset of stimulation. Analyses were based on the assumption that the activity of the neuron arose from a stationary process (cf. Perkel et al., 1967). Autocorrelograms were constructed from these data. Autocorrelations of normal intervals and shuffled intervals were then compared as a test of serial dependence and subjected to a chi-square test.

#### RESULTS

The responses of the sensory neurons in both the medial and the lateral sensillum can be divided into phasic and tonic periods (Figs. 1 and 3). Although the phasic period grades smoothly into the tonic, the phasic portion can generally be characterized as the recorded activity that occurs from time 0 to 100–150 ms after stimulus onset. Adaptation is very rapid during this period. Once the rate of firing has stabilized, it continues, under continuous stimulation, at that rate for 3–5 min. The maximum rate of firing is attained 25–85 ms after the onset of stimulation. Furthermore, only the cell giving the largest spike is active during this period (Figs. 1 and 2).

The tip-recording technique (Hodgson et al., 1955) used in these studies introduces certain artifacts that are largely unavoidable but which must be taken into consideration when analyzing records. For example, since the stimulus solution is the conductive path from the receptor to the silver electrode, different saps introduce different resistances into the circuit. Accordingly, recorded spike amplitudes vary depending on the stimulus, and the identity of spikes in a multineuronal response to a complex stimulus such as leaf sap can be ascertained in most cases only on a comparative basis. Thus, the cell producing the largest spike relative to the others does so in all normal stimulus situations. Sometimes analyses of spike waveform can add confirmatory information.

Characterization of a spike can also be confirmed in many cases by stimulating the sensillum with pure compounds, by mixtures of known pure compounds (e.g., sodium chloride and sucrose), and by mixtures of pure compounds and leaf sap (Dethier, 1973).

The “salt best” receptor in *Manduca* always gives the largest recorded spike (Schoonhoven and Dethier, 1966; cf. also Dethier and Kuch, 1971, and Dethier, 1973). Accordingly, in traces A–F in Fig. 1 the largest spike in the phasic period has been identified as the “salt best” cell even though it is not of the same absolute amplitude in all traces. Records of responses to 0.01 M NaCl are indistinguishable from responses to leaf saps in the phasic period (Fig. 2A). When plant saps are mixed, only this cell responds (Fig. 2D).

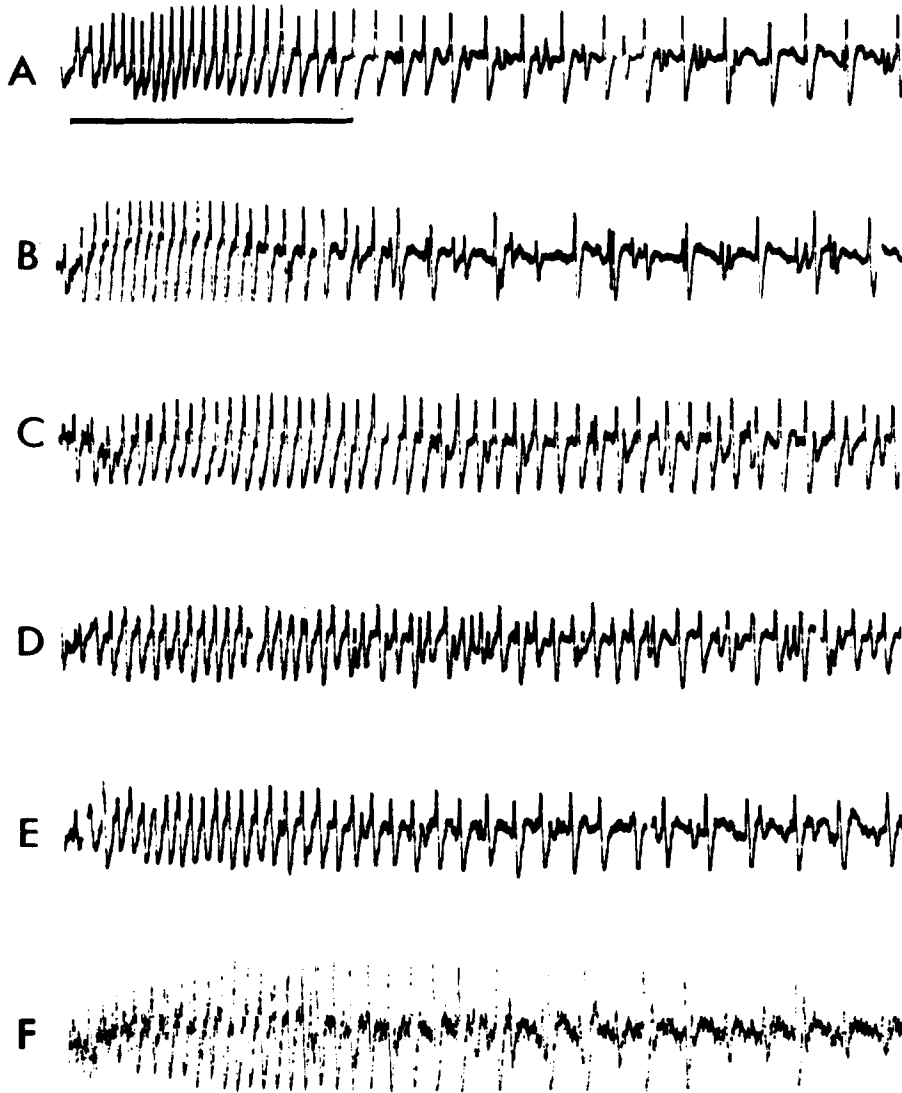


FIGURE 1. Responses of the medial sensillum during the first 300 ms of stimulation by tomato (A), tobacco (B), and Jerusalem cherry (C). Responses of the lateral sensillum to tomato (D), tobacco (E), and Jerusalem cherry (F). The large spike emanates from the "salt best" cell. The horizontal bar equals 100 ms. The record begins at time 0. Positive is up.

A second artifactitious feature in tip-recording is the frequent tendency of the spikes occurring during the first 20–50 ms, especially when the frequency is high, to exhibit a lower amplitude than later in the response (Figs. 1 and 2).

The tonic period can be said to begin at ~100–150 ms after the onset of

stimulation. Under continuous stimulation it usually lasts without appreciable changes up to 3–5 min. This period is characterized by its relative stability and by activity in three (occasionally four) cells including the one that fires during the phasic period (Fig. 3). The two cells giving spikes of medium and small amplitude respectively begin firing at ~150–200 ms after time zero.

There is considerable variation from one caterpillar to the next insofar as absolute rates of firing are concerned (as other workers have noticed; cf.

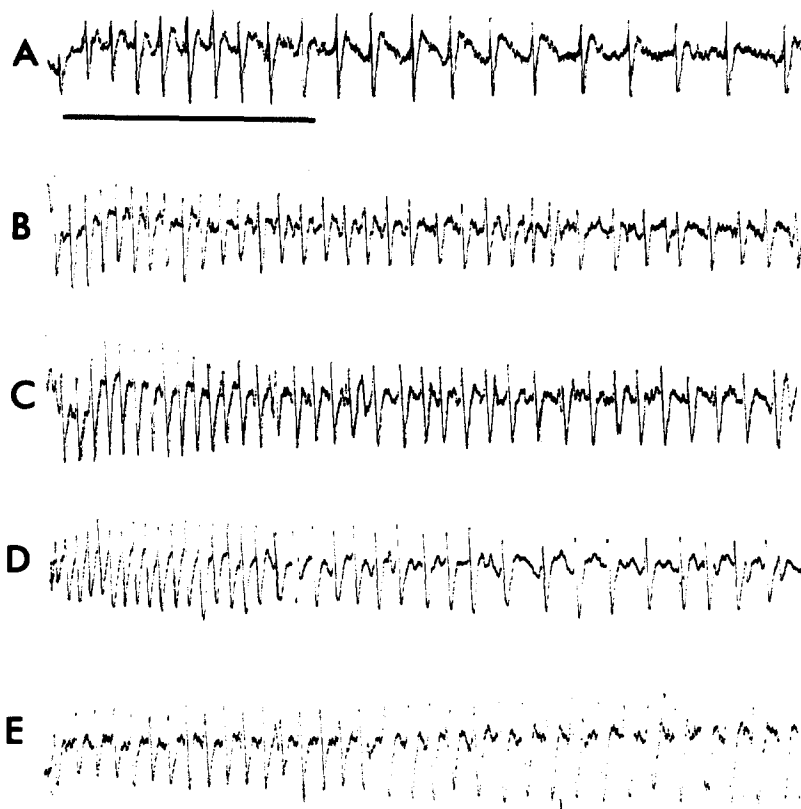


FIGURE 2. Responses of the medial sensillum to 0.01 M NaCl (A), carrot leaf sap (B), *Petunia* leaf sap (C), a 50/50 mixture of tomato and Jerusalem cherry leaf sap (D), and canna (E). Other details are as in Fig. 1.

Schoonhoven, 1976, 1977); however, each individual is internally consistent. For this reason, many of the comparisons described are those in which each caterpillar served as its own control. The relationships found with intra-individual comparisons were found to hold for all ten caterpillars.

The various saps evoked differential responses both in the phasic and the tonic periods. In the phasic period the only marked differences are the maximum rates of firing of the “salt best” cell and the rise and fall time of the spike train. Fig. 4 illustrates complete data averaged from five stimulations of

the medial sensillum of five animals with each sap. The relationships are the same for the lateral sensillum. Responses of the "salt best" cell to the unacceptable plant carrot and to the acceptable but toxic plant *Petunia* during the first 100 ms are indistinguishable from those of tomato (Fig. 2B and C).

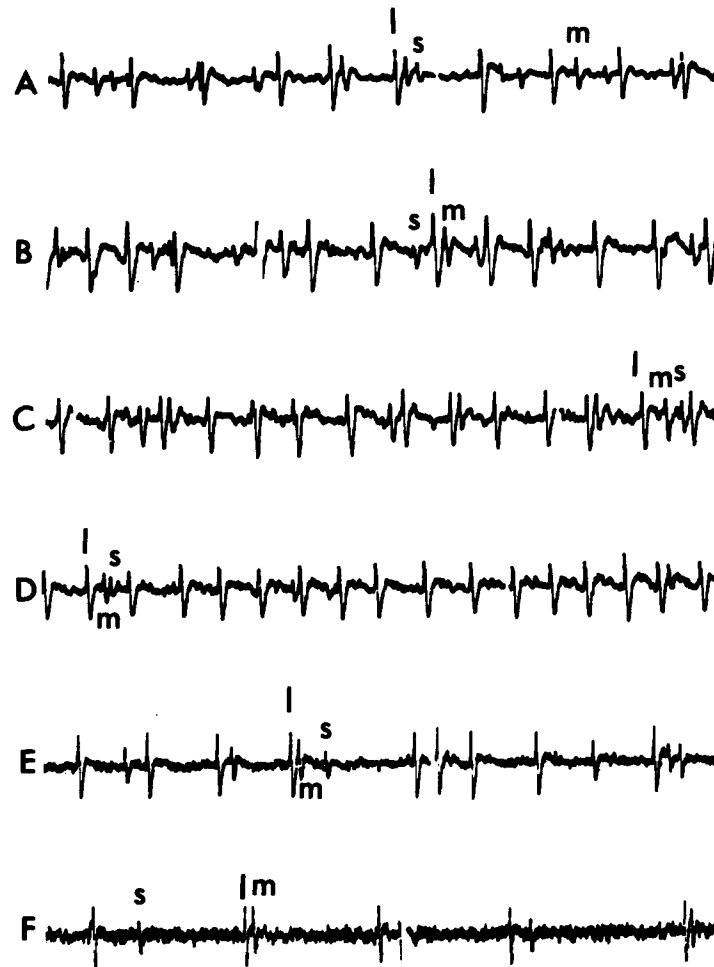


FIGURE 3. A representative 300-ms segment (the third second from time 0 + 200 ms) of the tonic response of the medial sensillum to tomato (A), tobacco (B), and Jerusalem cherry (C). Response of the lateral sensillum to tomato (D), tobacco (E), and Jerusalem cherry (F). Horizontal bar equals 100 ms. Three of the four classes of spikes are shown: L = largest, the "salt best" cell; M = medium-sized; S = small.

During the first 30 ms of phasic responses tomato differs significantly from Jerusalem cherry (Duncan's Multiple Range Test,  $P < 0.01$ ) but not from tobacco. During the initial 30 ms tobacco and Jerusalem cherry are not significantly different. At 50 ms there is no significant difference among the

saps. From 75–200 ms Jerusalem cherry is different ( $P < 0.01$ ) from the other two plants. In general, except for the fact that the peak rate of response to tomato (at 30 ms) is higher than that to tobacco, the responses to these two plants do not differ.

In the tonic period the dominant spike is that of the “salt best” cell. The distribution of the intervals in the firing pattern from the medial sensillum during the third second is depicted in Fig. 5. The data are averaged from five replications on each of five caterpillars. The means, standard deviations,

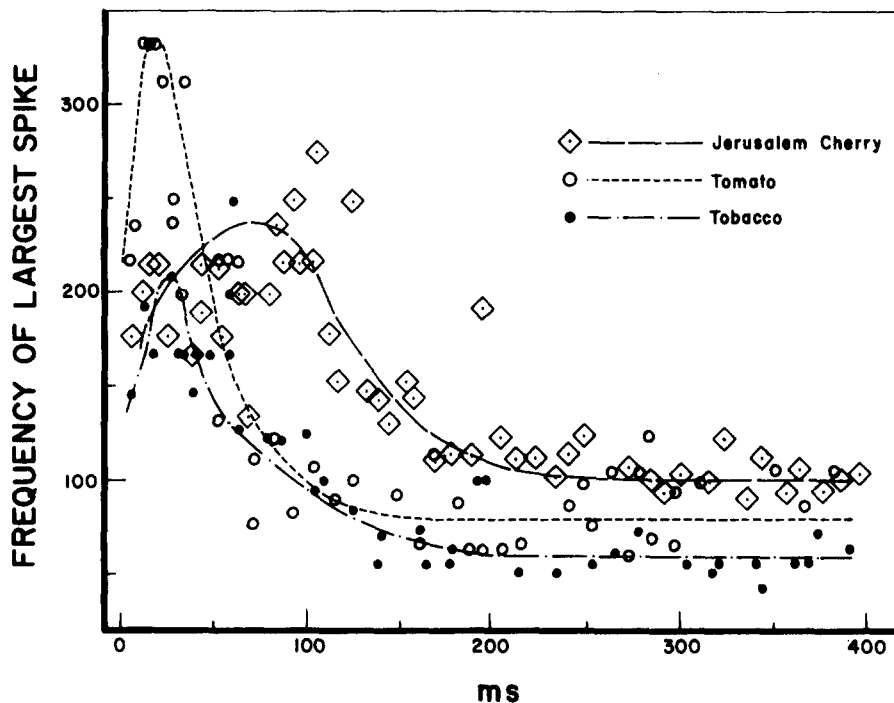


FIGURE 4. Comparison of the instantaneous frequency of the largest spike (from the “salt best” cell) from the medial sensillum during the first 400 ms of stimulation by each of the three leaf saps. Each point represents the combined average of five stimulations of five animals. The frequency is the reciprocal of the interspike interval.

variances, and coefficients of variation are as follows: tomato: 10.12, 3.37, 11.0, 0.33; tobacco: 14.94, 3.94, 15.1, 0.26; Jerusalem cherry: 10.97, 2.56, 6.40, 0.23. A Kolmogorov-Smirnov  $z$  sample test indicated that the distribution of intervals for tobacco differs from those of the other two plants at  $P < 0.01$  and that tomato is not significantly different from Jerusalem cherry.

A similar analysis of the firing patterns of the medium-sized spike (Fig. 6) gave the following values for means, standard deviations, variances, and coefficients of variation, respectively: tomato: 17.18, 10.88, 115.87, 0.63; tobacco: 21.10, 10.92, 116.37, 0.51; Jerusalem cherry: 21.0, 10.27, 101.46, 0.48.



The Kolmogorov-Smirnov test indicated that the distributions of tomato and tobacco are not significantly different ( $P > 0.1$ ), but tomato does differ from Jerusalem cherry ( $P = 0.01$ ).

If temporal patterning occurs in the neural response to any of the leaf saps, it can be detected by measuring the higher-order interspike intervals as they occur in their normal sequence and then comparing this sample with higher-order intervals obtained after the sequence of spikes in the same section of the train has been randomized. Such comparisons of autocorrelations of unshuf-

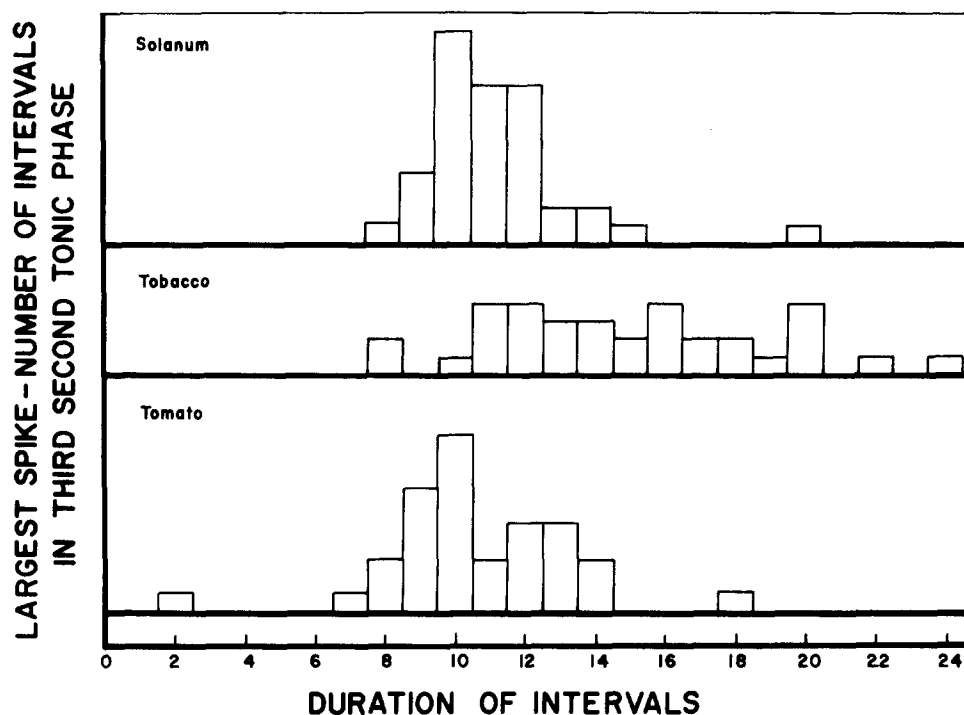


FIGURE 5. Distribution of the intervals of the largest spike from the medial sensillum during the third second (from time 0 + 200 ms) of response to each of the three leaf saps. Data are averaged from five stimulations of five caterpillars. Each point on the abscissa equals 2 ms.

fled and shuffled data for each of the three saps revealed no significant difference in the cases of tomato ( $P > 0.25$ ) and tobacco ( $P > 0.5$ ) (Fig. 7) but a difference in the case of Jerusalem cherry ( $P < 0.005$ ) (Fig. 8).

The total number of spikes fired by each of the cells in each sensillum during the third second of continuous stimulation are compared in Fig. 9. The means are derived from five stimulations of each of four caterpillars. Absolutely and relatively the relationships among the three classes of spikes are similar in the lateral and medial sensilla. The largest spike tends to be the most frequent, followed by the medium-sized spike; however, for the grouped data the difference between the two classes of spikes is not significant.

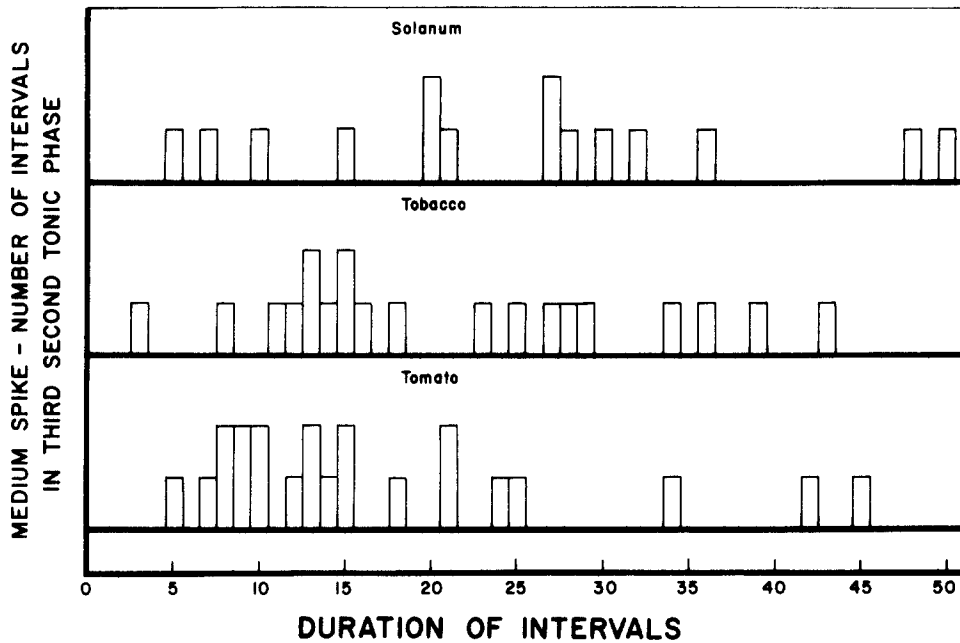


FIGURE 6. Distribution of the intervals of the medium spike of the medial sensillum. Other details are as in Fig. 5.

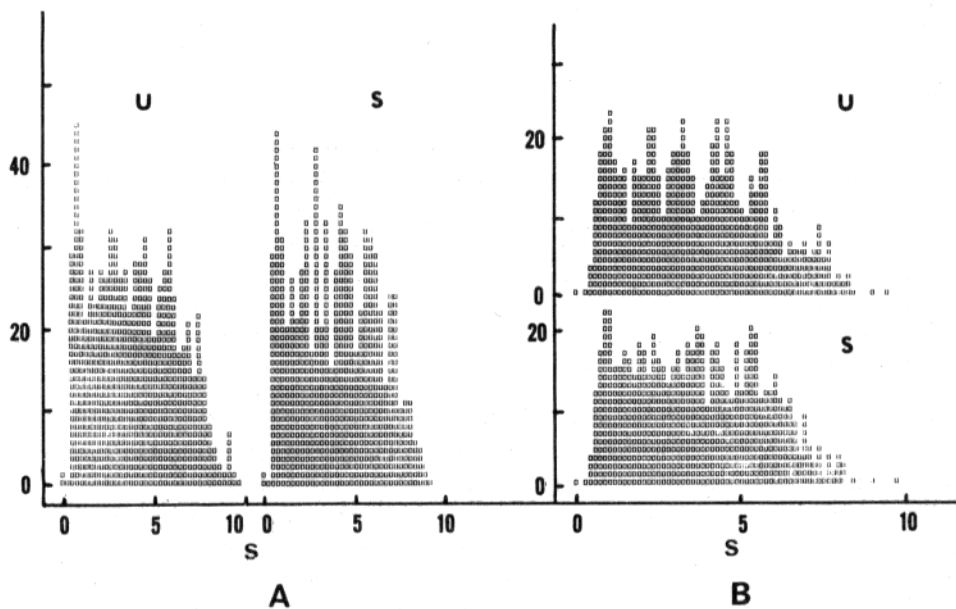


FIGURE 7. Unshuffled and shuffled autocorrelograms of intervals of the largest spike during 10 s from time 0 + 200 ms for tomato (A) (200 spikes) and tobacco (B) (140 spikes). Data are from one animal. Time on abscissa indicates total elapsed time. Ordinate indicates the number of occurrences.

When comparisons are made within an animal (five stimulations with each sap), most animals show a significant difference between the large spike and other classes ( $P = 0.001 - 0.05$ , Mann Whitney U,  $n = 5$ ) although exceptions do occur (cf. tomato preparation 7, Fig. 10). The medium spike differs from the other two classes less often (cf. tobacco preparation 3, Jerusalem cherry

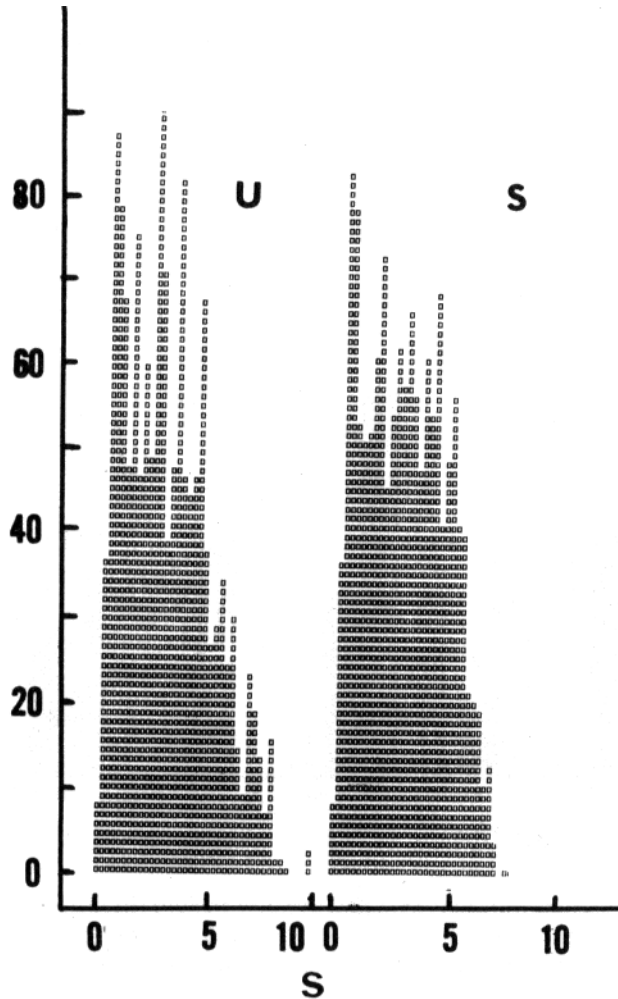


FIGURE 8. Same as Fig. 7 for Jerusalem cherry (260 spikes).

preparations 6 and 7). The most consistent and striking phenomena are the significant differences ( $P = 0.001 - 0.05$ ) among all classes of spikes with Jerusalem cherry and the very low frequency of the smallest spike.

Comparisons of the relative discharge magnitudes among the three cells in response to different concentrations of tomato sap and Jerusalem cherry sap showed no consistent change from the situation obtained with undiluted sap. This is illustrated by data obtained with tomato (Figs. 11 and 12). At all

concentrations except 25% the large spike differs significantly from the small one ( $P = 0.01$ ) but not from the medium one.

Behavioral reaction times of ten caterpillars to deterrents (0.001 M caffeine and 0.001 M quinine monohydrochloride averaged 310 ms (SD, 9.53). The shortest recorded time was 200 ms. Behavioral observations indicated that not less than 5 s are required for any overt sign of discrimination among acceptable plants. This information was obtained as follows. As a caterpillar is chewing the edge of a leaf upon which it has been induced, another leaf is edged into position so that the next sweep of the mandibles encounters the new leaf.

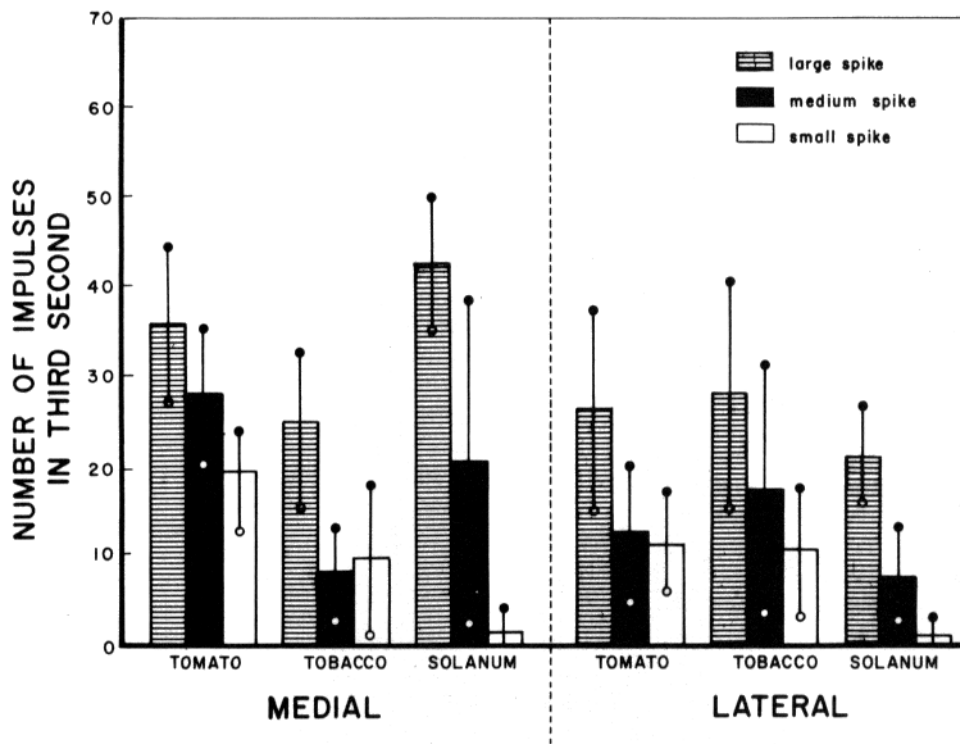


FIGURE 9. Total numbers of spikes fired by each of three cells in each sensillum during the third second of continuous stimulation by each leaf sap. Vertical bars represent  $\pm 1$  SD. Data are averaged from five stimulations of each of four caterpillars.

When the new leaf is the same species as the one being eaten, there is no interruption in the repetitive biting-chewing sequence. When the new leaf is a different species, the biting-chewing sequence continues for a short period and then stops. The shortest time recorded in a sample of ten caterpillars was 5 s, the longest was 16 s.

#### DISCUSSION

Detailed behavioral studies of tobacco hornworms (*Manduca sexta*) have shown that these caterpillars are capable of distinguishing acceptable from unac-

ceptable plants and discriminating among different acceptable species on the basis of information received via the gustatory system. Furthermore, caterpillars can be trained to prefer a particular acceptable plant over another (Jermy et al., 1968), and this preference can be reversed. Hanson and Dethier (1973) have shown that these preferences can be induced even when larvae are deprived surgically of all chemosensory input except that from eight receptor neurons in each of the paired maxillae and a pair of three in the epipharynx.

Since the epipharyngeal receptors appear to be concerned only with moni-

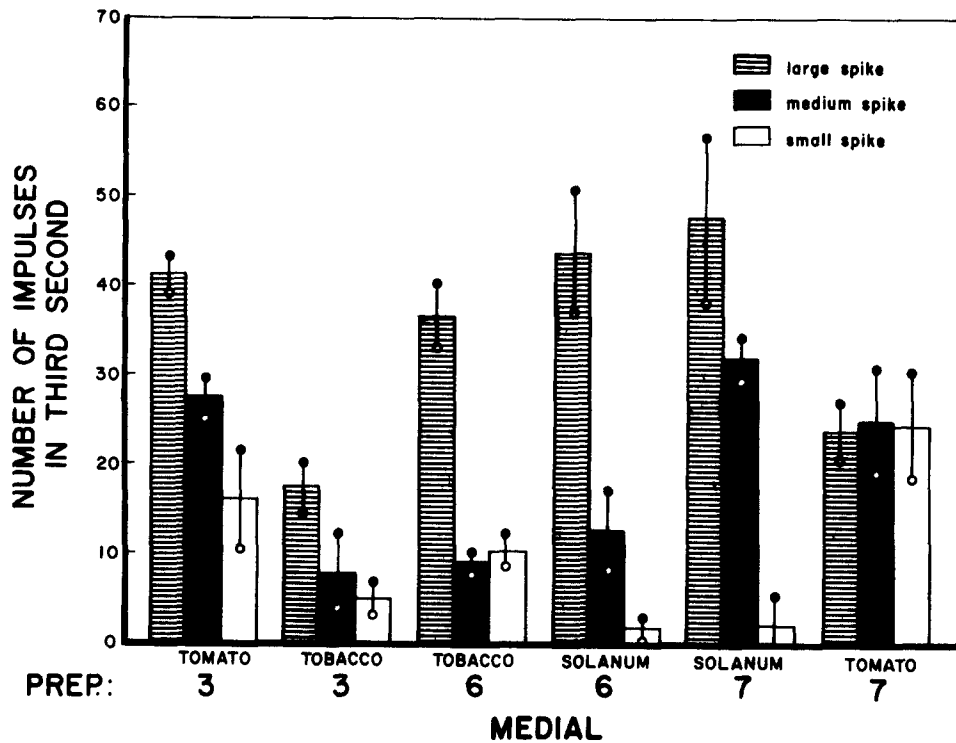


FIGURE 10. Same comparisons as in Fig. 10 but within each of three individual caterpillars (preparations 3, 6, and 7). Symbols are as in Fig. 9.

toring swallowing (Ma, 1972, 1976; de Boer et al., 1977), and because there is evidence in other insects that selection can be made before food has entered the preoral region (Le Berre, 1970), it has been concluded that eight cells of each maxilla provide adequate information to enable *Manduca* larvae to distinguish among food plants. Previous studies, plus the data presented here, indicate that these cells respond both to acceptable and unacceptable plants (Dethier, 1973). In the present study eight cells were indeed involved, but only six of these responded electrophysiologically with a high enough frequency and amplitude to allow meaningful analyses.

For purposes of this investigation, detailed knowledge of the gustatory breadth of specificity of each neuron and of the chemical constituents of the leaf saps, although desirable, was not crucial.

Earlier investigations do provide some information on the specificity of the receptors (Schoonhoven and Dethier, 1966; Schoonhoven, 1972). Each one is categorized in terms of the compound that stimulates it best, that is, elicits the highest frequency of neural discharge at the lowest concentration. Thus, the "salt best" receptor, to use the terminology often applied to mammalian gustatory fibers (cf. Nowlis and Frank, 1977), or salt receptor for short, does not respond exclusively to sodium chloride but responds best to it. On this basis the maxillary receptors of *Manduca* can be identified as follows. In the

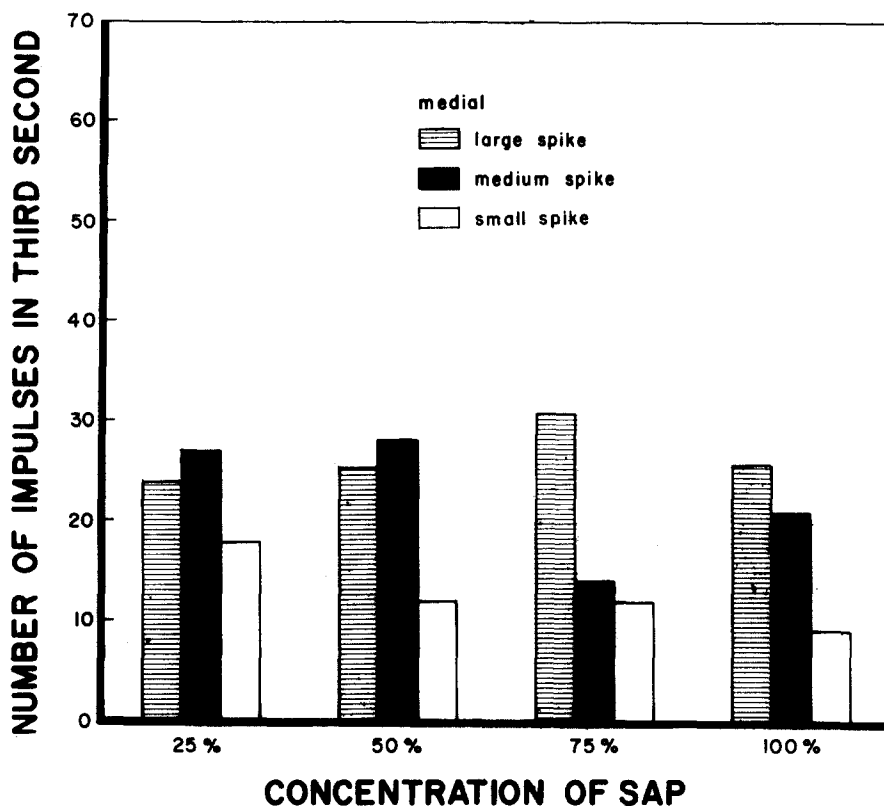


FIGURE 11. Responses of three cells of the medial sensillum of one caterpillar to dilutions of tomato leaf sap.

medial sensillum the four are: salt, sugar, inositol, and alkaloids; in the lateral sensillum they are salt, sucrose/glucose, inositol, and salicin.

Beyond the fact that the leaf saps contain salts, carbohydrates, amino acids, glucosides, and alkaloids (e.g., tomatin, solanine, nicotine), few details of the chemical composition are known.

Consideration of the combined data from all behavioral and electrophysiological studies to date leads to the conclusion that at least three of the eight maxillary receptors can separately provide information that is able to trigger acceptance or rejection (cf. Schoonhoven, 1972 for review). When pure sugar

(sucrose, fructose, or glucose) is placed on the sensillum, only the sugar receptor responds by virtue of its specificity, and it evokes a feeding response from animals that are water satiated but food deprived; that is, water is refused and sugar is accepted. Similarly, when inositol is placed upon the sensillum, only the inositol receptor responds, and inositol is accepted. When salicin is placed on a receptor, the salicin receptor responds, and an animal that is water or food deprived refuses to accept either water or sugar adulterated with salicin.

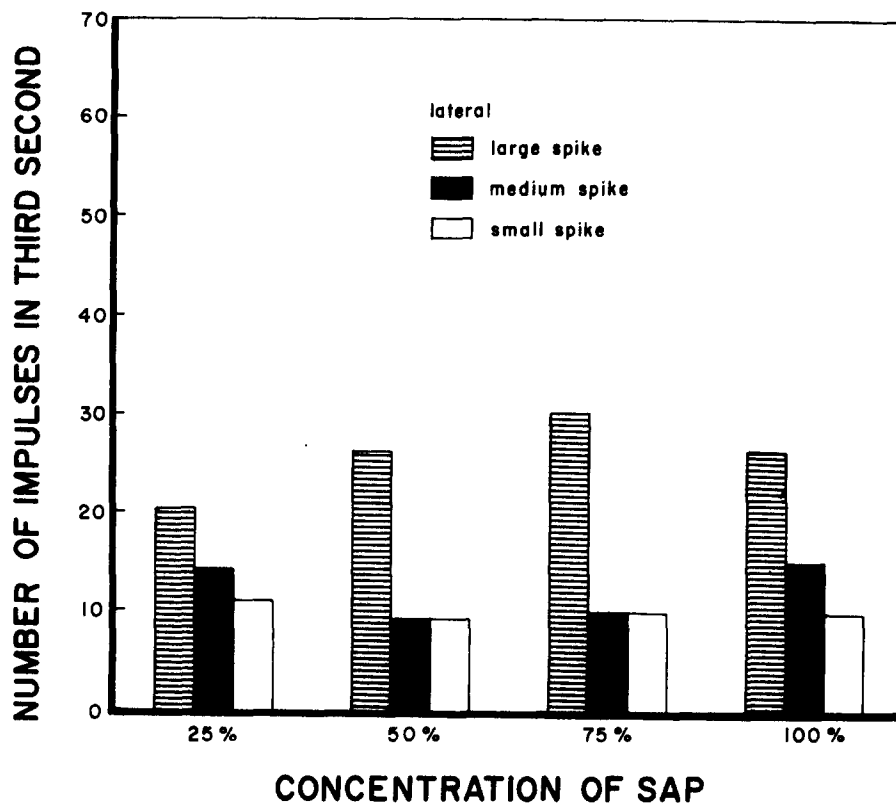


FIGURE 12. Same comparisons as in Fig. 11 for cells of lateral sensillum.

In the instances just described the individual receptors are acting as “labeled lines”; the nature of the code is clear. Furthermore, since only one cell is responding in each case, the question of assigning information transmittal to the phasic or to the tonic portion of the receptor response is not critical. In any case, the behavioral response times that have been measured for a yes or no answer accord favorably with the time of occurrence of the phasic electrophysiological response. The shortest time measured for alkaloids was 200 ms.

Some idea of the significance of this time may be gained by reference to more detailed studies conducted with blowflies. The shortest total reaction time of the blowfly from the instant of tarsal stimulation with sucrose to

proboscis extension, measured cinematographically, is 100 ms (Dethier, 1955). The shortest total time calculated from electrophysiological measurements of time from stimulus onset to the first muscle potential (40 ms) plus the time from the first muscle spike to proboscis extension (20 ms) is 60 ms (van der Starre and Ruigrok, 1980). The shortest time recorded by Getting (1971) from the first effective sensory spike of the sugar cell in the labellum to the first motor spike is 2.56 ms. The shortest time from the onset of stimulation of the sugar cell in the labellum to proboscis extension is 80 ms (Dethier et al., 1965). With stimulation of the salt cell the time is 113 ms, of which 80 ms is the time from stimulus onset to the first muscle spike and 33 ms is the time from the first motor spike to proboscis movement (Dethier, 1968). Rough calculations show that total reaction time is on the order of 100 ms, of which 2.56–80 ms is the time from stimulus onset to the first motor spike (this time depends upon spike frequency) and 20–33 ms is the time from motor activity to movement.

If times are comparable in caterpillars, the total reaction time allows for less than 150 ms of message to arrive in the central nervous system before behavior ensues.

In all of these cases the only decision to be made was “yes” in the case of flies and “no” in the case of caterpillars. Thus, there is ample time for the phasic portion of the maxillary sensory response to convey specific information via a labeled line, and indeed only one receptor is required for this task. If the first and only cell to fire in this interval is a sugar cell, a positive feeding response would be triggered. If the cell is a deterrent cell, rejection would be triggered. On the other hand, it is conceivable that the phasic response serves merely to alert the central nervous system to the fact that information is to follow.

In the study reported here, the only cell that responds in the phasic period to tomato, tobacco, and Jerusalem cherry is the “salt best” cell. This cell also responds in the phasic period to the unacceptable plants carrot and canna and to the toxic plant *Petunia*. Mixing the leaf saps, for example, tomato and Jerusalem cherry (Fig. 2D), gives a response that combines the rapid rise time seen with tomato alone with the more delayed peak obtained with Jerusalem cherry, but only the one cell responds. Furthermore, the electrophysiologically recorded phasic response to any of the three plants can be mimicked with the appropriate concentration of sodium chloride.

Thus, although the rise and fall times with Jerusalem cherry differ from those with tomato and tobacco and could provide a means of distinguishing Jerusalem cherry from the other two plants, neither a rate of change code nor a spatial representation (labeled line) during the phasic period can provide unambiguous information to permit the fine discrimination observed behaviorally.

More detailed information presumably necessary for making fine distinctions among such complex mixed stimuli as leaf saps probably requires more time and more cells. Although Halpern and Tapper (1971) have shown that a rat can make gustatory discriminations within 200 ms of tasting solutions,



neurophysiological details necessary to relate this accomplishment to the mechanism of coding are not at hand. All things considered, it appears unlikely that a rate code or a spatial representation (labeled lines) in the phasic period of sensory response is involved in quality discrimination of complex mixtures.

We look then to the tonic period to provide this information. Behavioral observations had indicated that not less than 5 s is required for any overt sign of discrimination among acceptable plants. This time clearly falls within the tonic phase of the electrophysiological response.

At least three cells of each sensillum are firing during this period. Given this multineuronal response, several methods of coding information are possible. Of the 32 candidate neural codes listed by Perkel and Bullock (1968) as being supported by some physiological evidence, four codes in addition to labeled line coding already discussed seem particularly relevant to the case of *Manduca*. These are: single-unit variability codes; single-unit temporal pattern codes; an ensemble code (across-fiber patterning); and a combination of the foregoing three.

The comparisons of the mean spike intervals, standard deviations, coefficients of variation, and variances of the "salt best" cell (averaged from five replications on each of five caterpillars) responding to each of the plant saps during the third second show that tobacco differs from tomato and Jerusalem cherry but that tomato and Jerusalem cherry are not significantly different. These relationships also hold when 10-s samples are compared and when comparisons are restricted to individual caterpillars serving as their own controls.

A similar comparison involving the medium spike shows that tomato and tobacco are not significantly different nor are tobacco and Jerusalem cherry; however, tomato does differ from Jerusalem cherry. Thus, insofar as a variability code is concerned the "salt best" cell differentiates tobacco from the other two plants, and the cell giving the medium-sized spike differentiates tomato from Jerusalem cherry. These two cells transmitting simultaneous information to the central nervous system could therefore provide a basis for distinguishing the three plants.

It is a well-known fact that variability in intervals is often a function of rate of firing, the variation being less at higher rates than at low (Ratliff et al., 1968). Tobacco, evoking the largest variability from the "salt best" cell is also the stimulus eliciting the lowest rate during the tonic period. The cells producing the medium-sized and small spikes fire at an even lower rate, and they have the highest variability.

The possibility of a temporal patterning code is indicated by an examination of autocorrelations. Discrepancies between shuffled and unshuffled data indicate serial dependence (Moore et al., 1966). Thus, with Jerusalem cherry there is an indication of a temporal pattern in the spike train, whereas none is indicated for tomato and tobacco.

The total pattern of firing of the three cells of each sensillum studied here offers another potential code. Although data presently available do not

support the idea that tomato and tobacco can be distinguished from each other on an ensemble ratio of firing rate basis (behavioral data are ambiguous on this point; Jermy et al., 1968), they do reveal a means of distinguishing Jerusalem cherry. With this plant the ratio of the frequency of the small spike to the other two is invariably smaller than is the case with tomato and tobacco.

For an ensemble code to be successful, the firing characteristics of the cells contributing to the ensemble would have to differ with different stimuli, that is, either the means, variances, or both would have to differ from one stimulating sap to the next. This is clearly the case with Jerusalem cherry, where for both the large and small spike the variance is low.

The possibility of there being interspike dependence is minimized by the observation that ratios of firing rates among the three cells are different with the different saps. Furthermore, a careful examination of the occurrence of the medium-sized and small spikes during the tonic period reveals no constant association (cf. Fig. 3). The presumed lack of dependence accords well with what is known about insect gustatory sensilla in general. In the most thoroughly studied insect, the black blowfly, no electrophysiological effect of one chemoreceptive neuron on another has ever been reported, nor are there any anatomical connections between peripheral cells and axons in any insect studied (Dethier, 1976c).

One of the difficulties with the across-fiber patterning hypothesis is the potential ambiguity that can be introduced by the effect of concentration of stimulus on rate of firing. It was for this reason that measurements were made of responses to different concentrations of leaf saps. Under natural conditions leaf saps do not fluctuate between the extremes tested here (25–100%). It is all the more interesting, therefore, to find that there is no significant change in the pattern (the simultaneous magnitudes of discharge from each of three cells) such that alterations in concentrations of a sap can cause that sap to mimic others. It is probable that the different cells have different concentration/response spectra such that under normal fluctuations of concentrations of leaf saps the relationships of an across-fiber pattern are preserved. It is also possible that the thresholds are such that the receptor cells are already saturated at low concentrations of sap and have approached their maximum rates of firing. That this may be true is indicated by the concentration response curve of the "salt best" cell during its phasic period. For each of 10 caterpillars tested there was no significant difference in firing rate of this cell over the 25–100% range of concentration of tomato sap.

Of the candidate codes examined here, some evidence has been found to support the role of one in the phasic period and three in the tonic period. In the phasic period labeled lines can signal acceptance or rejection. There is no evidence that there is a labeled line for tomato or tobacco or Jerusalem cherry as such or for any chemical specific to any one of these plants (as for example, sinigrin in *Cruciferae* and the sinigrin receptor in *Pieris* caterpillars). Future chemical analyses of the saps may force modification of this statement.

In the tonic period some discrimination among plants would be possible on

the basis of a variance code in two of the cells studied or a temporal patterning of firing in response to Jerusalem cherry. The fact that the response to all saps involves multineuron activity argues strongly in favor of an ensemble code. At the peripheral level necessary prerequisites of this code would be differences in means and/or variances of spike intervals of the cells in response to different stimuli. Thus, variance might have no meaning in itself but only in its contribution to the ensemble.

Although it is possible that different coding mechanisms could be used to encode different qualities, the data presented here provide evidence for a single code which relies on the across-fiber temporal pattern of action potential production. This conclusion is based primarily on the demonstration that other possible codes cannot account for all the data.

Clearly a number of parameters of the receptor neuron discharge patterns have been found to vary with the nature of the stimulus which in turn affects overt behavioral response. As Uttal (1969) has cautioned, not all parameters of sensory discharge patterns are important contributors to codes. Furthermore, information may be ignored or modified at higher levels. *Manduca* provides a model system for further peripheral and central analyses of these desiderata.

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