Olfactory Receptor Responses to Sex Pheromone Components in the Redbanded Leafroller Moth

ROBERT J. O'CONNELL

From The Rockefeller University, New York 10021

ABSTRACT Electrical responses of single olfactory receptor neurons of the male redbanded leafroller moth were elicited by each of the principle components of the sex pheromone and six other behaviorally active compounds. Response frequencies to equal intensities of each of these compounds and changes in response frequency with increasing amounts of any one compound, varied from receptor to receptor. These differences in response characteristics appear to be due to factors intrinsic to the olfactory receptor neuron and not to factors external to it. The encoding of odor quality by these receptor neurons cannot be in the simple presence or absence of activity in any one of them. Rather, odor quality may be encoded by the pattern of activity which invariably arises across an ensemble of receptor neurons, each having its own distribution of sensitivities.

INTRODUCTION

The ability to discriminate a large number of different odors is a characteristic feature of olfactory systems. As a result, the encoding of odor quality[1] by olfactory receptor neurons has long been a central issue in research on olfaction. Electrophysiological studies of vertebrate systems have revealed that individual olfactory receptor neurons respond to a range of different compounds which are known to evoke different odor qualities in man. These effective odor spectra have been shown to vary considerably between different receptor neurons (Mathews, 1972; O'Connell and Mozell, 1969; Altner and Boeckh, 1967; Gesteland et al., 1965; Gesteland et al., 1963). It has also been shown that equal amounts of neural activity can be elicited in a single vertebrate receptor neuron by several different odor compounds by simply adjusting their relative odor intensities. Therefore it has been concluded that the absolute amount of neural activity in a single receptor neu-

[1] Two chemicals are said to differ in odor quality if they elicit different behaviors when each is presented to an organism at equal, gas phase, intensities.

ron cannot provide a unique code for a single odor quality (O'Connell and Mozell, 1969). In consideration of the observed response properties, it has been postulated that quality coding in vertebrate olfactory receptor neurons must be a cooperative venture among a large number of receptor neurons resulting in a singular pattern of activity across this ensemble of neurons for each discriminable odor (O'Connell and Mozell, 1969).

In studies of the insect olfactory system, as exemplified by *Bombyx mori*, the domesticated silk moth, Schneider and his colleagues have found two types of olfactory receptor neurons (Kaissling, 1971; Schneider, 1969, 1965; Schneider and Steinbrecht, 1968). The first type has been termed a "generalist." Neurons of this type are often found in sensilla basiconica and respond to a variety of flowery and aromatic odors in a manner analogous to that just described for vertebrate receptor neurons. The second type of insect olfactory receptor neuron as described by Schneider for *Bombyx* has been termed a "specialist." "Specialists" are usually found in sensilla trichodea; they respond to compounds which evoke a specific behavioral response, such as the synthetic sex pheromone *trans*-10, *cis*-12-hexadecadien-1-ol acetate. These neural responses have been shown to occur in a highly stereotyped and reproducible way (Boeckh, 1969; Boeckh et al., 1965). For example, each of over 500 individual specialist receptor neurons recorded from *Bombyx* were noted as having an *identical* response spectrum when stimulated with Bombykol (the synthetic sex pheromone) and several of its related isomers (Priesner, 1969). Threshold responses for the various synthetic isomers of Bombykol required stimulus intensities two to six log steps more intense than those needed for the natural pheromone. In all cases compounds which were found to excite the Bombykol specialists (i.e. those compounds which constitute the response spectrum for these receptor neurons) were found to elicit the sex behavior of the male (Priesner, 1969).

Receptor neurons with identical specificities and in which excitation evokes a fixed unit of behavior are defined as part of a "labeled line" system of quality coding. In a labeled line system a particular odor quality is encoded by the simple presence of activity in that single receptor neuron (Mountcastle, 1968). Under this scheme, each discriminable odor quality would require its own type of specialist (labeled line), each with its own unique and nonoverlapping response spectrum. In *Bombyx*, Bombykol is apparently the only naturally occurring sex pheromone compound. Consequently, a single class of sex pheromone specialists appears to be sufficient in this animal.

In contrast, there is a considerable amount of behavioral evidence which indicates that the sex pheromone systems of other insects are more complex than that found in *Bombyx* (Borden, 1974; Hirai et al., 1974; Roelofs and Cardé, 1974; Bartell and Roelofs, 1973; Beroza et al., 1973; Cardé et al., 1973; Kaae et al., 1973; Minks et al., 1973; Klun and Robinson, 1972;

Ganyard and Brady, 1971; Roelofs and Comeau, 1971 *a*; 1968). Such is the case with the redbanded leafroller moth (RBLR), *Argyrotaenia velutinana.* After the isolation and identification of the primary component of the RBLR sex pheromone, *cis*-11-tetradecenyl acetate (*c*-11-TDA) by Roelofs and Arn (1968), it was found that a number of structurally related compounds could modulate its effectiveness in field trapping experiments (Roelofs and Comeau, 1968, 1971 *a*). For example, the addition of 10 μg of *trans*-11-tetradecenyl acetate (*t*-11-TDA) the geometric isomer of the primary component, to a trap baited with 10 μg of *c*-11-TDA reduces the catch of males nearly 90% below the number which would have been trapped with *c*-11-TDA alone (inhibition). On the other hand, 10 μg of dodecyl acetate (DDA) added to a similar trap baited with 10 μg *c*-11-TDA increases the catch 170% (synergy). Out of the 46 different natural and synthetic compounds which have been field tested, 21 were found to be without effect (no modulation of response), 16 were synergistic, and the remaining 9 were inhibitors.

Further complexities in the organization of the RBLR pheromone system were noted in a recent field study which demonstrated that large numbers of male RBLR were attracted to traps baited with a mixture containing 92% *c*-11-TDA and 8% *t*-11-TDA. Changes in this ratio, in favor of either compound, resulted in a pronounced decrease in trapping efficiency (Klun et al., 1973). On the other hand, 10-fold increases in trapping efficiency were obtained by the addition of two parts of DDA to each part of the attractive isomeric mixture (Roelofs et al., 1975). These three compounds (*c*-11-TDA, *t*-11-TDA, and DDA) have now been identified in the effluvia collected from virgin RBLR females indicating that they are components of the natural pheromone system (Roelofs et al., 1975).

It should be apparent that additional levels of complexity in the organization of the RBLR pheromone system will be uncovered as behaviors other than those paramount in field trapping experiments (i.e. oriented flight toward and ultimately into a trap) are measured. For instance in a laboratory bioassay Bartell and Roelofs (1973) have shown that male RBLR can respond differentially to highly purified samples of *c*-11-TDA and *t*-11-TDA. In particular a cartridge loaded with 1 μg of *c*-11-TDA elicits rapid walking accompanied by wing vibration, brief periods of flight, and copulatory attempts. However, an equal amount of *t*-11-TDA elicits only "a short-lived wing 'buzzing' response." The fact that equal amounts of these two compounds can elicit different behavioral sequences in laboratory bioassays even though neither compound traps males in field tests indicates that these compounds are individually perceived by RBLR males as two different odor qualities.

In an electroantennogram (EAG) study by Roelofs and Comeau (1971 *b*) most of the compounds used in their field testing program produced an elec-

trical response in the male RBLR antenna. Consequently, the antenna must contain olfactory receptor neurons which are responsive in some way to each of these compounds. In addition, they observed that the magnitude of the EAG responses to the primary sex pheromone component and its geometric isomer were two to three times larger than those obtained with any of the other compounds. However, there was no correlation between the magnitude of the EAG and the type of field trapping behavior elicited by a compound. Because of the summated nature of the EAG technique, it was not possible to obtain information about the coding mechanisms utilized by single receptor neurons.

In a preliminary electrophysiological study of single RBLR receptor neurons it was determined that there are two olfactory receptor neurons located in each of the individual sensilla trichodea, each neuron producing discriminable action potential heights and each clearly responsive to both *cis*- and *trans*-11-tetradecenyl acetate (O'Connell, 1972). These compounds were without effect on the olfactory receptor neurons found in a limited sample of male RBLR sensilla basiconica. Responses to other behaviorally active compounds were not systematically investigated.

The olfactory-initiated behavior elicited in the RBLR moth by *c*-11-TDA and certain structurally related compounds, including its geometric isomer, appears to be more complex than that elicited in *Bombyx* by Bombykol and its isomers. In particular, there are 25 compounds, each related to *c*-11-TDA, which can modulate the field attractancy of the primary component of the RBLR sex pheromone. Modulation of the attractancy of Bombykol by related compounds and the necessity for precise isomeric ratios has not been reported in *Bombyx*. Since the behavioral response of an organism is determined first by its sensory capabilities, it is possible that the considerably more complex behavioral situation in RBLR requires a more complex coding scheme at the level of individual olfactory receptor neurons. This additional complexity might arise in one of several ways: (*a*) the number of different specialist receptor neurons may be greater, since an additonal one would be required for each discriminable odor compound, or (*b*) the receptor neurons that respond to the primary sex pheromone component and its modulators may be similar to those seen in vertebrates where quality coding involves the collective action of many types of receptor neurons, each with its own unique but overlapping odor spectrum, or (*c*) the receptor neurons may have some combination of properties intermediate between those described in *a* and *b*.

The present study examined several different parameters of single olfactory receptor neuron activity. These include: (*a*) a quantitative description of the levels of spontaneous activity for receptor neurons both within and between individual sensilla, (*b*) the responses elicited in these neurons by graded intensities of both *cis*- and *trans*-11-tetradecenyl acetate, and (*c*) a qualitative

determination of the response spectra of individual receptor neurons to a number of different behaviorally active compounds. These data should provide a foundation for the ultimate understanding of quality coding in insect olfactory receptor neurons.

MATERIALS AND METHODS

Animals

RBLR larva were cultured in closed containers containing an alfalfa leaf meal diet at 25°C and continuous light. The media is a modification of Redfern's (1964) and contains 1,500 g alfalfa leaf meal, 400 g sucrose, 75 g yeast extract, 25 g sorbic acid, 20 g methyl *p*-hydroxybenzoate, and 2 liters water. Pupae were removed from the culture dishes thrice weekly, sexed, and the males which were to be used for recording isolated from all contact with RBLR females or their pheromones. The remainder of the pupae were placed in a mating cage which was maintained at 22°C and with a normal light cycle. Eggs were collected and hatched according to the techniques of Glass and Hervey (1962). Individual culture dishes containing 25–30 g of media were sown with 15–20 newly hatch larva and placed in the incubator. The time of eclosion for each experimental male was usually noted to the nearest half day. These experimental animals were subjected to normal laboratory temperatures and light cycles with ad-lib access to 5% dextrose in water.

Adult males were prepared for recording by first anesthetizing them with CO_2, placing them on their dorsal surface in a closely fitted holder, and restraining their head and legs with drops of molten wax (Fig. 1 A). This prevented all but antennal movements. After this immobilization, the animal was allowed to recover from the effects of the CO_2. During the recovery period (20–30 min), the scales bridging the joints of the 40 antennal segments were carefully removed with a camel's hair bristle dipped in molten wax. This exposed the long sensillar trichodea located at the proximal margin of each segment (Fig. 1 D). Most of the successful recordings were obtained from these trichodeal sensilla. The entire antenna (ca. 3 mm long) was then fixed to a platform, whose surface was covered with an adhesive, and positioned so that its leading edge, with its profusion of sensory sensilla, could be explored with the recording microelectrode. A small metal rod (125 μm in diameter) was carefully pressed against the junction of the antennal flagellum and pedicel to prevent movement of the antenna (Fig. 1 A).

Recordings

Electrodes were constructed from 125-μm diameter tungsten wire electrolytically sharpened to tip diameters of less than 0.5 μm and plated with platinum black (Gesteland et al., 1959). The electrodes were carried in high power (× 800) micromanipulators (E. Leitz Inc., Rockleigh, N. J., model 520–137) and were positioned under optical control (× 600). The ground electrode was inserted into the blood space of one of the 10 most distal antennal segments (Fig. 1 C). The recording electrode was positioned at the base of any one of the sensilla trichodea which are distributed over the 30 more proximal segments. Fig. 1 D shows the typical orientation of the recording

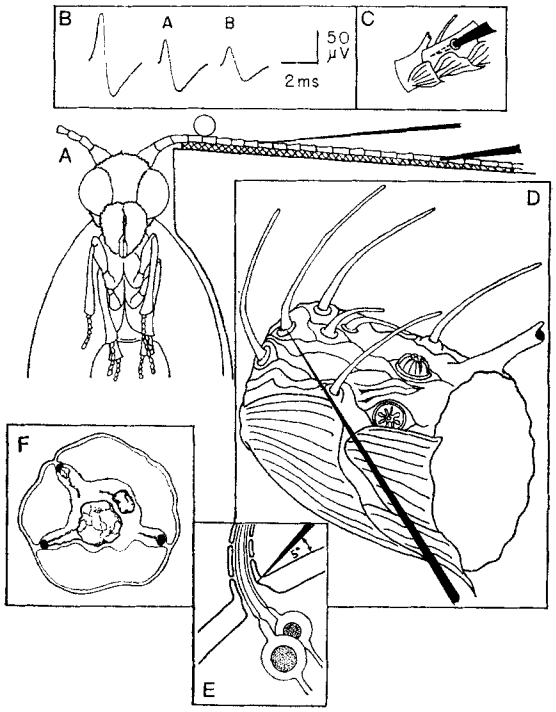

FIGURE 1. Schematic diagram of a male redbanded leafroller moth, the experimental arrangement and the anatomy of trichodeal sensilla. (A) The experimental animal is held in a Plexiglas holder by drops of wax (not shown) on the mouth parts, legs, and wing margins. A portion of the animal's left antennae is shown affixed to the Plexiglas platform with a layer of adhesive (hatched area) and held in place with a 125 μm tungsten rod (circular cross section). The indifferent electrode is usually placed approximately 10 segments distally from the location illustrated here. (B) Tracings of the action potentials obtained from a sensillum trichodeum. The relative differences in peak-to-peak amplitude between the three potentials are normally larger than the ones shown here. However, the differences in waveform are fairly typical. Only the receptor neurons which produce the action potentials labeled A and B are olfactory receptor neurons. (C) Detail showing the insertion of the indifferent electrode into the blood space of one of the distal 10 antennal segments. This insertion is usually made through one of the coeloconica sensilla normally found in the distal portion of each segment. (D) Surface view of one antennal segment showing the usual placement of the recording microelectrode. The distal margin of the segment with its prominent blunt-tipped peg is shown to the right. The leading edge of the antennal segment, i.e. that portion normally facing into the air stream when the animal is flying, is shown uppermost. Odor stimuli are normally directed towards this edge. The recording electrode is inserted into the base (diameter of 2 μm) of one of the long sensilla trichodea (50–100 μm). Just to the right are two of the short sensilla basiconica (15–50 μm) which contain olfactory receptor neurons which are not responsive to sex pheromones. To the right of these are two stiff sensilla which may contain mechanoreceptors as judged by their specialized attachment to the surface of the cuticle. Further to the right are two sensilla coeloconica. The function of these sensilla is unknown but they do provide convenient places for the insertion of ground electrodes. The blunt-tipped peg appears to have a large opening

electrode with respect to the base of a single trichodeal sensillum. The geometric constraints imposed by the design of the insect holder and the micromanipulator prevented exploration of the entire circumference of the sensillum by the recording electrode. Normally only the quadrant shown in the figure (1 D) could be probed.

Gentle pressure, and tapping of the micromanipulator carrying the recording electrode, in a direction parallel to the electrode's long axis, usually resulted in penetration of the sensillum cuticle (Fig. 1 E) and registration of trichodeal receptor neuron spike activity (Fig. 1 B). This electrical activity was amplified with an AC-coupled amplifier with gain of 10,000 and band pass of 0.14–5.3 kHz. The amplified spikes were displayed on an oscilloscope, monitored with an audiomonitor, and processed on-line with a digital computer (O'Connell et al., 1973).

The spike amplitude recorded from each of the three trichodeal neurons increases as the recording electrode is moved deeper into the sensillum. Unfortunately, the deeper penetrations suffer the risk of damaging one or more of the receptor neurons. Consequently, electrode penetration was halted as soon as the amplitude of the smallest spike exceeded 50 μV. In this electrode position the action potentials recorded from the three receptor neurons have characteristic differences in spike waveform and amplitude (Fig. 1 B). These differences were used by the computer system to identify, separate, time, and store the train of action potentials produced by each of the receptors in the sensillum (O'Connell et al., 1973).

Stimulation and Data Reduction

All compounds used as stimuli were synthesized in the laboratory of Dr. W. Roelofs of the Cornell University, New York State Agricultural Experiment Station, Geneva, N. Y. Approximately 10 μg of each compound was analyzed with a Hewlett-Packard model 5750B gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a flame ionization detector, and a glass column (2.4 m by 4 mm ID) packed with 3% FFAP[2] on 80/100 mesh Chromosorb W. The temperature of the column was maintained at 200°C, and the helium flow rate was 30 cc/min. Under these conditions all compounds were found to be better than 99% pure. Cross-contamination between stimulus compounds was not apparent. Where appropriate, the isomeric purity of the compounds was verified on thin layer chromatography plates coated

[2] Carbowax 20 M-Terephthalic Acid terminated.

at its tip and might therefore contain taste receptor neurons. The number of sensilla shown here has been greatly reduced for purposes of illustration. However, the relative placement of the individual sensilla and the electrode is correct. (E) Schematic representation of a section through a sensillum trichodeum showing the placement of the recording microelectrode and the inferred differences between the diameter of the two receptor neuron dendrites. (F) Schematic representation of a cross section through a sensillum trichodeum, again illustrating the difference in dendrite diameter. Also shown are three pores with associated pore tubules through which stimulus molecules apparently diffuse. Individual odor molecules presumably interact with specialized portions of the dendritic membrane (receptor sites) and cause a modulation in the rate of action potential production. Fig. 1 E and F have been adapted and redrawn from electron micrographs of Steinbrecht (1973).

with silica gel G and impregnated with 20% $AgNO_3$. After spotting, the plates were eluted with benzene and then visualized with 0.1% 2,7-dichlorofluoresceine in isopropanol. Under these conditions no isomeric impurities were detected.

A range of stimulus intensities for each compound was produced by serial dilutions with light mineral oil (National Formulary). The dilution series varied in 0.5 $\log_{10}$ steps from 316 $\mu g/\mu l$ to 0.316 $\mu g/\mu l$ and was verified with the gas chromatograph (vide supra). One μl of the desired dilution was placed on a piece of filter paper (1 cm^2) carried within a glass odor cartridge (Becton-Dickinson & Co., Rutherford, N. J. model 2546 Baketel adapter). The cartridge had an outlet diameter of 4 mm and an internal volume of 0.2 cc. In all cases reference to a particular odor intensity refers only to the amount of chemical loaded into the odor cartridge and not to the amount actually arriving at the individual receptor neurons. Control cartridges held filter papers loaded with 1 μl mineral oil. Between experiments each odor cartridge was stoppered with a Teflon plug and stored in the dark in a closed container at 4°C.

The chemicals used as stimuli were drawn from among those behaviorally active compounds identified in field trapping experiments by Roelofs and Comeau (1971 *a*). The compounds were the primary pheromone component *cis*-11-tetradecenyl acetate (*c*-11-TDA); four of the behavioral inhibitors of this compound: *trans*-11-tetradecenyl acetate (*t*-11-TDA), tridecyl acetate (TriDA), *cis*-11-tetradecenyl-1-ol (*c*-11-TDol), *cis*-11-tetradecenyl formate (*c*-11-TDF); and four of the behavioral synergists: dodecyl acetate (DDA), *cis*-7-dodecenyl acetate (*c*-7-DDA), 10-propoxydecanyl acetate (10-PDA), and 10-undecenyl acetate (10-UDA).

During the experiments the desired odor cartridge was connected via Teflon tubing (Hamilton Co., Reno, Nev., no. 86509) to the normally closed arm of a three-way Teflon valve. The onset and duration of air flow through this arm, and thus through the odor cartridge, was controlled by the digital computer and timed to an accuracy of 100 μs. The odor cartridge was held in a jig carried on a micromanipulator. This allowed the cartridge's outlet to be reproducibly placed, at an angle of 45° from the perpendicular, exactly 7 mm above the surface of the antenna and centered on the tip of the recording microelectrode. The normally open arm of the valve was used as an interstimulus purge line. It was connected via Teflon tubing to another glass cartridge whose outlet was positioned opposite the odor cartridge so that the purge air stream swept across the antenna, toward the outlet of the odor cartridge. This arrangement effectively prevented any stimulus molecules which might diffuse out of the odor cartridge during the interstimulus interval from ever reaching the antenna. It also ensured that the stimulus waveform issuing from the odor cartridge had relatively sharp leading and trailing edges.

A vacuum line positioned 10 cm below the animal exhausted air at a flow of 2.2 liters/min, preventing the buildup of contaminating odors in the general environs of the insect. Air for the odor and purge lines was obtained from a tank of air zero gas (hydrocarbon impurities less than 2 ppm) which was further purified by passing it through $CaCl_2$, activated charcoal, and silica gel before delivering it to the inlet of the three-way valve. The flow rate in the purge line was 111 cc/min and that in the odor line was 52 cc/min.

After successful placement of the recording electrode, an estimate of the physi-

ological range of stimulus intensities to be used was obtained by first stimulating with the 31.6 $\mu g/\mu l$ dilution of either *cis*- or *trans*-11-tetradecenyl acetate. If the response elicited by this intensity was in the range 100–150 impulses/10 s, it was taken as the upper limit of stimulus intensity. In those sensilla where this intensity produced responses less than this range, stimulus intensity was raised in 0.5 log unit steps until more than 100 impulses were elicited or until the maximum stimulus intensity of 316 $\mu g/\mu l$ was attained. In all cases the remaining stimuli were then given in order of decreasing intensity. In experiments where several different odor compounds were used, their order of presentation was randomized. Control stimuli (cartridge loaded with 1 μl mineral oil) were interspersed with odor stimuli on a random basis except that they were always given whenever the odor compound was changed.

For each stimulus presentation 20 s of neural activity was processed. This time consisted of a 10-s prestimulus interval followed by 1 s of stimulation and then by a 9-s poststimulus interval. A minimum of 2 min 40 s then elapsed before another stimulus sequence was begun. These restrictions on both the maximum stimulus intensity and the minimum interstimulus interval were implemented to avoid the deleterious effects on unit activity which can be obtained in vertebrate olfactory receptors with too intense or too frequent stimuli (Shibuya and Tucker, 1967; Gesteland et al., 1963).

For each receptor neuron in a sensillum, the computer produced counts of the number of action potentials in the 10-s prestimulus interval and in the succeeding 10 s. The difference between these two frequencies, expressed in impulses/10 s, was used as a quantitative measure of response. In those rare occasions when a control stimulus produced a small response, its value was subtracted from the responses obtained with the preceding odor stimuli.

RESULTS

General Characteristics

A microelectrode inserted at the base of a sensillum trichodeum usually records the electrical activity produced by three different receptor neurons, as judged by the three different action potential amplitudes and waveforms encountered (Fig. 1 B). These action potentials are initially positive, diphasic spikes with durations of 2.5–3.0 ms and peak-to-peak amplitudes of 50–750 μV. The receptor neuron which produces the largest amplitude spike fires infrequently (15–20 impulses/h) and is apparently not an olfactory receptor; at least, I have been unable to find a compound which will stimulate it. Consequently, the activity of this receptor neuron will not be considered further. The remaining two receptor neurons in the sensillum are olfactory receptors which respond to a number of different odors including the primary component of the female sex pheromone, *c*-11-TDA. The olfactory receptor neuron which produces the larger amplitude spike will be referred to as unit A, the one producing the smaller amplitude spike will be referred to as unit B (Fig. 1 B, Fig. 3).

Spontaneous Activity

The mean spontaneous activity for each of 52 individual receptor neurons (26 pairs of A and B units), was computed from an average of 19 separate 10-s estimates per neuron. The distribution of these means in both receptor neurons is skewed positively toward the lower levels of spontaneous activity (Fig. 2). The median and its standard error for mean spontaneous activity in the sample of A units was 26.4 ± 6.7 impulses/10 s; whereas, the value for the sample of B units was 18.9 ± 5.3 impulses/10 s. Thus, the level of spontaneous activity in the A unit of a sensillum was usually slightly higher than that of its companion B unit. This difference is statistically significant (Wilcoxon matched pairs signed-ranks test: $Z = -1.79$; $P < 0.05$; Siegel, 1956).

There were also differences in the general levels of spontaneous activity between cells in different sensilla. The positive correlation (Spearman's rank correlation coefficient: $r_s = +0.65$; $P < 0.001$; Siegel, 1956) between the absolute levels of spontaneous activity in these two neurons across sensilla indicates that the two cells in a sensillum tend to have similar relative levels of spontaneous activity when compared with other sensilla.

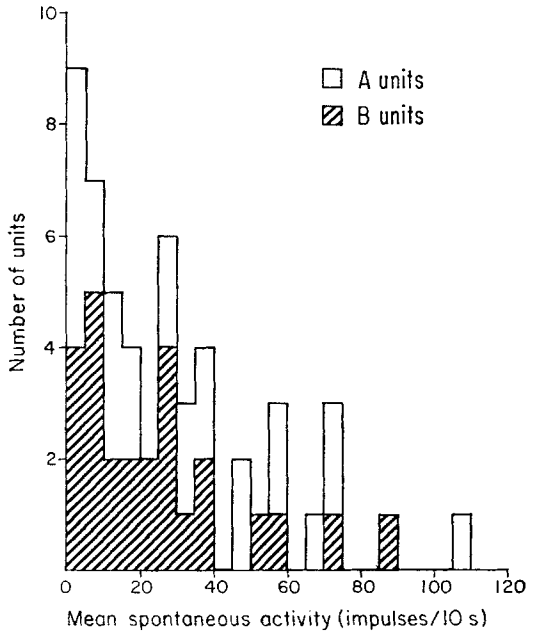

FIGURE 2. A frequency distribution of the mean levels of spontaneous activity in 52 olfactory receptor neurons (26 pairs). Receptor neurons are accumulated into spike frequency bins 5 impulses wide, i.e., there were 5 A units and 4 B units whose mean spontaneous activity fell between 0 and 5 impulses/10 s.

Response to cis- and trans-11-Tetradecenyl Acetate

Fig. 3 shows the electrical activity recorded from a sensillum trichodeum during stimulation with an intensity series of *c*-11-TDA. Both units A and B respond with an increase in response frequency to this compound. These

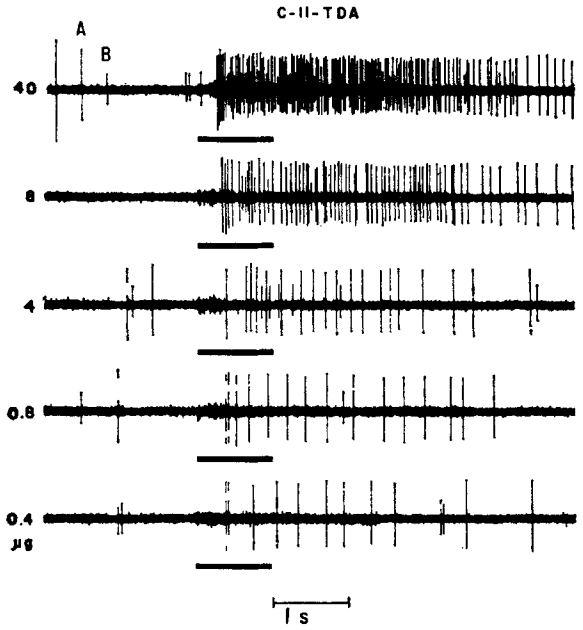

FIGURE 3. Electrical responses recorded from a single sensillum trichodeum of the male redbanded leafroller moth. Responses were elicited by stimulation with graded intensities of the primary component of the sex pheromone *cis*-11-tetradecenyl acetate (*c*-11-TDA). Stimulus duration of 1 s is indicated by the length of the bar under each record. The action potentials produced by three individual neurons are seen in the prestimulus interval of the top record (40 μg). The first and largest amplitude spike occurs infrequently and apparently does not represent the activity of an olfactory receptor. The remaining two receptor neurons respond to odors and have been arbitrarily labeled the A and the B units solely on the basis of differences in the relative sizes and shapes of the action potentials that they normally produce.

responses are largely tonic with an initial phasic component at higher stimulus intensities. At all intensities the responses usually continue for a time after the end of stimulation (3–8 s). Typically, response frequency decreases and response latency increases as stimulus intensity is lowered.

For most receptor neurons, response frequency approximates a linear function of the log of stimulus intensity. Response functions (plots of response frequency versus log stimulus intensity) for *c*-11-TDA are shown in Fig. 4. Each curve represents the responses of one receptor neuron. In this sample

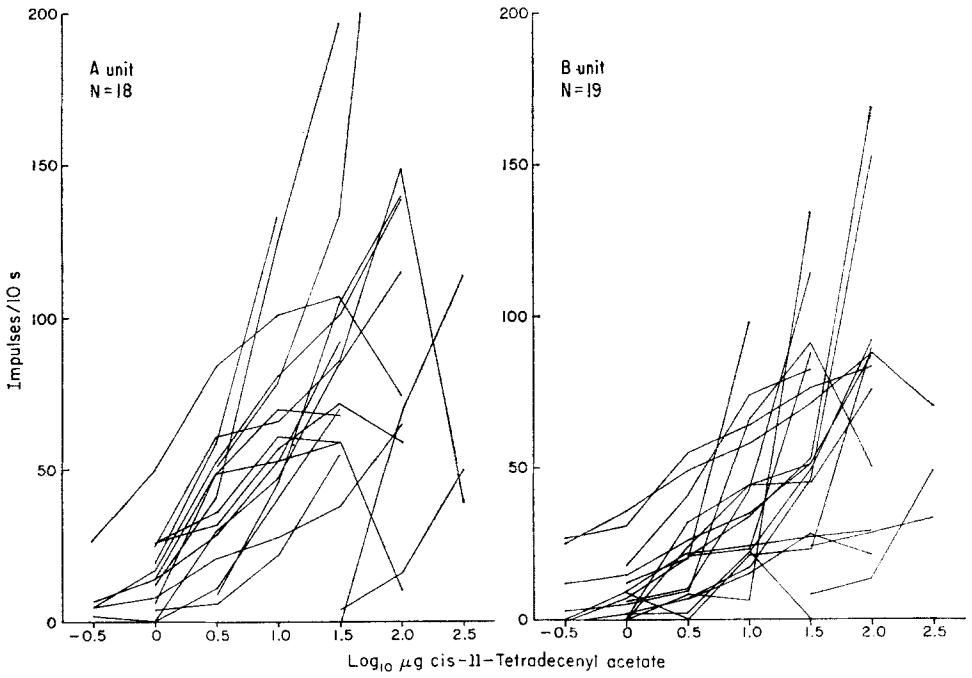

FIGURE 4. Response functions for the indicated number of A and B units. These include 17 pairs of receptor neurons each obtained from a separate sensillum. The abscissa is the number of μg of *cis*-11-tetradecenyl acetate (*c*-11-TDA) loaded into the odor cartridge and not the amounts actually arriving at the receptor neurons.

of A and B units, there are 17 receptor neuron pairs each recorded from a separate sensillum plus one (in A) or two (in B) receptor neurons whose companion died before the complete intensity series was presented. On inspection of Fig. 4 it is apparent that individual receptor neurons differ among themselves with respect to a number of parameters. These include the minimum stimulus intensity necessary for a specified threshold response, the rate at which response frequency increases with further increases in stimulus intensity (response slope), the absolute size of the maximum response (response maximum), and the stimulus intensity required to elicit that maximum. It is also apparent that the A units often have steeper response functions and higher response maxima than the B units for this compound. In several of the receptor neurons (four A's and three B's), response frequency decreased at the highest stimulus intensity presented.

The response functions elicited in the same 17 pairs of receptors (plus one A and three B units) by stimulation with *t*-11-TDA, the geometric isomer of the primary pheromone component, are shown in Fig. 5. There are again considerable differences among receptor neurons with respect to the various response parameters. Contrary to the case for *c*-11-TDA stimulation, the

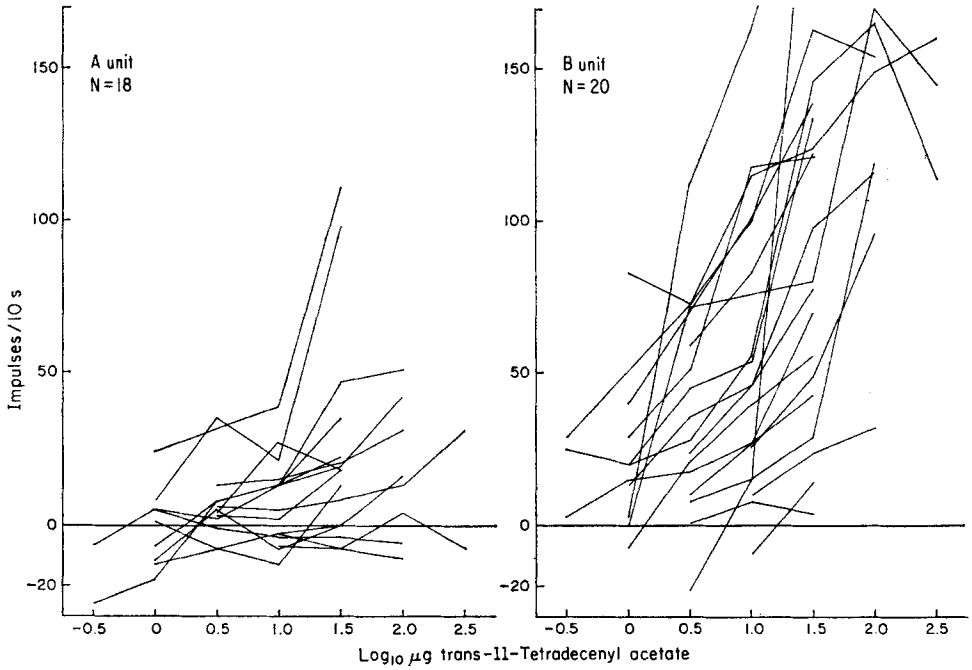

FIGURE 5. Response functions obtained by stimulating the 17 pairs of receptor neurons shown in Fig. 4 with *trans*-11-tetradecenyl acetate (*t*-11-TDA). Responses which are less than 0 impulses/10 s are ones in which the level of spontaneous activity in the 10-s period before stimulation was higher than the driven activity observed in the subsequent 10-s period.

sample of B units with *t*-11-TDA stimulation display steeper response functions and higher response maxima than the sample of A units. A distinct feature of these response functions is the appearance of some inhibitory responses (decreases in response rate) to *t*-11-TDA. A larger proportion of A units show such inhibitory responses (9 out of 18) than do B units (3 out of 20). None of these receptor neurons were inhibited by stimulation with *c*-11-TDA. Again some of the receptor neurons (two A's and four B's) show decrements in response frequency at the higher stimulus intensities. As noted previously, the maximum stimulus intensity applied to a particular receptor neuron varied between sensilla and was ultimately constrained by a desire to prevent deleterious effects on unit activity. Consequently, the proportion of decrementing responses observed in both these samples may underestimate the percentages that would have been observed in the population in the absence of such constraints.

The response parameters for *c*-11-TDA and *t*-11-TDA stimulation are summarized in Table I which lists the ranges observed in both A and B units. The slopes of the response functions and their X intercepts were obtained by

TABLE I
RANGES FOR RESPONSE PARAMETERS

	c-11-TDA		*t*-11-TDA	
	A units	B units	A units	B units
Slope of the response function (*impulses/10 s*)	22.7–171.2	9.0–92.8	−4.0–60.4	7.0–231.0
X intercept of the response function (log_{10} μg)	−1.14–1.49	−1.56–1.42	−1.13–1.09	−1.21–1.20
Response maximum (*impulses/10 s*)	59–≥318	22–≥169	5–≥111	8–≥239
Stimulus intensity at response maximum (log_{10} μg)	1.0–≥2.5	1.0–≥2.5	0–≥2.5	1.0–≥2.5

computing a straight line fit with the method of least squares for each of the response functions plotted in Figs. 4 and 5. In every case the correlation coefficient relating receptor neuron response to the log of stimulus intensity had an absolute value larger than 0.80 (Hays, 1963). The slope of the response function can be taken as a measure of the gain or sensitivity of an individual receptor whereas the X intercept may be regarded as an estimate of its threshold. Statistical analysis of both these parameters revealed that units A and B produce responses, when stimulated with either *c*-11-TDA or *t*-11-TDA, whose slopes are drawn from two different distributions ($P \leq 0.02$; Wilcoxon Matched Pairs Signed Rank Test; Siegel, 1956). On the other hand the X intercepts for both units are drawn from a single distribution. Identical findings were obtained when the responses of a single receptor neuron, either A or B, were compared across the two stimuli (for response slope $P \leq 0.05$, X intercepts not significant).

In many of the receptor neurons the values for the response maxima and intensity at maximum were undetermined because the upper levels of stimulus intensity were often not given in order to avoid deleterious effects on receptor neuron response. In these cases the largest values actually obtained were used instead. Consequently the ranges listed in Table I must underestimate the span of actual ranges which should appear in the population. In spite of this restriction, each of the variables listed in Table I exhibits a relatively broad range of response values. This is indicative of the overall variability seen in the responses of one receptor neuron when compared to those of another.

In spite of this variability, simple inspection of Figs. 4 and 5 along with Table I indicates that there are also some general trends in the data. To facilitate a comparison of these trends the mean responses for each individual sample were computed. The four resulting mean response functions

are plotted in Fig. 6 (i.e., averaging all the functions of Fig. 5 A yielded the bottom function in Fig. 6). As suggested by the individual response functions, the average A unit has a steeper response function and a higher response maximum to *c*-11-TDA than does the average B unit; *t*-11-TDA produces the opposite result, a steeper response function and a higher maximum in the B

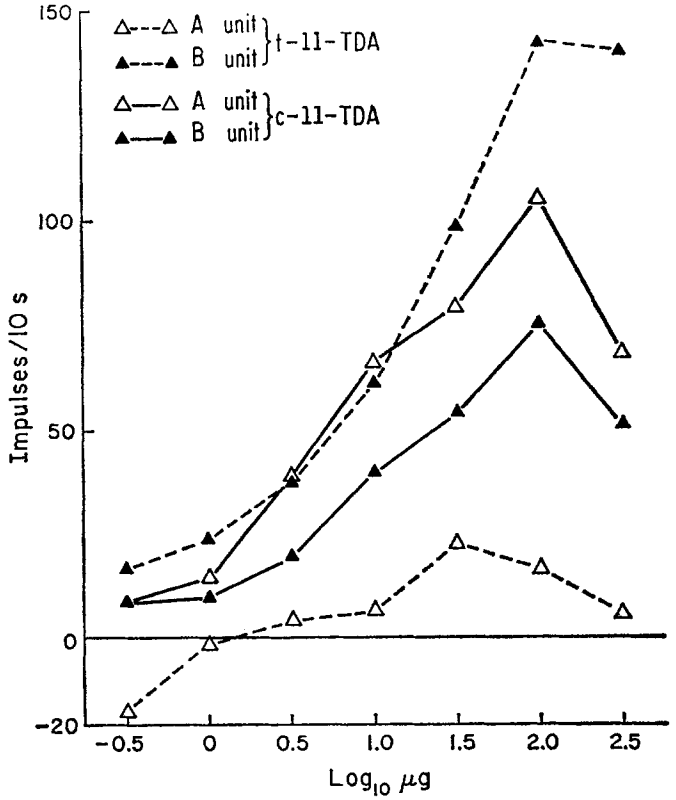

FIGURE 6. The average response functions of both A and B units when stimulated with *cis*- or *trans*-11-tetradecenyl acetate. The response function at the top of this figure was obtained by calculating the arithmetic mean of the individual responses illustrated in Fig. 5 B at each of the indicated stimulus intensities.

unit. Above 10 μg, the B unit response to *t*-11-TDA exceeds the A unit response to *c*-11-TDA. For each level of stimulus intensity, the B unit produced larger responses (B > *A*) to the *trans*-compound, whereas, the A unit produced larger responses (A > B) to the *cis*-compound. In the case of unit A the response to *t*-11-TDA is an actual inhibition at lower intensities.

These trends actually represent the responses of individual receptors for these two compounds. They do not arise as a result of averaging together units with different absolute levels of response. For instance stimulation with 31.6 μg/μl of *c*-11-TDA produced responses whose order was A > B in 83%

of the sensilla sampled and stimulation with an equal amount of *t*-11-TDA produced responses whose order was B > A in 87% of the sensilla.

Responses to Other Biologically Active Compounds

The two receptors of a sensillum trichodeum produce responses which are quite variable in absolute terms but which nonetheless maintain a reasonably constant relationship with one another when stimulated with either *c*-11-TDA or *t*-11-TDA. Would such be the case with other behaviorally active compounds? Seven additional compounds, selected from among those having biological activity in field tests (Roelofs and Comeau, 1971 *a*), were used as stimuli for six pairs of receptor neurons. A qualitative estimate of the responses elicited by these compounds along with both *cis*- and *trans*-11-tetradecenyl acetate is contained in Table II. Responses were elicited in one or more of the receptor neurons sampled by each of the compounds when they were presented at a stimulus intensity of 31.6 μg/μl. However, the relative effectiveness of the compounds varied both within and between sensilla. For instance, both units A and B in sensillum 1 respond similarly to all compounds except when stimulated with the cabbage looper (*Trichoplusia ni*) sex pheromone, *cis*-7-dodecenyl acetate, in which case the B unit was inhibited and the A unit was excited. On the other hand, the pattern of responses elicited

TABLE II

RESPONSES OF RBLR OLFACTORY RECEPTOR NEURONS TO BEHAVIORALLY ACTIVE CHEMICAL COMPOUNDS*

			Inhibitors				Synergists			
Sensillum	Unit	Attractant, *c*-11-TDA	*t*-11-TDA	TriDA	*c*-11-TDol	*c*-11-TDF	DDA	*c*-7-DDA	10-PDA	10-UDA
1	A	+	+	0	0	+	+	++		
	B	+	+	0	0	+	+	−		
2	A	++	+	−	−	+	−	−	+	0
	B	++	++	+	0	++	0	0	+++	+
3	A	+++	0	0	−	+	−	0		
	B	+	+	−	0	−	−	+		
4	A	+++	+			−			0	+
	B	+++	+++			+			+	0
5	A	++	+			+	+	0	+	
	B	0	0			+	+	−	+	
6	A	++	+	−	0	+	+	−	−	+
	B	++	++	+	0	0	+	+	++	+

KEY: $R \leq -6 = -$; $-5 \leq R \leq 5 = 0$; $6 \leq R \leq 50 = +$; $51 \leq R \leq 100 = ++$; $R \geq 101 = +++$; Blank = not tested

R response magnitude in impulses/10 s.

* All compounds presented at an intensity of 31.6 μg/μl.

in sensillum 2 differs from that seen in sensillum 6 for all compounds except *cis*- and *trans*-11-tetradecenyl acetate and tridecyl acetate.

For many of the receptor neurons sampled, *c*-11-TDA was the most effective stimulus (7 neurons out of 12). With the exception of tridecyl acetate, 10-undecenyl acetate and *cis*-11-tetradecenyl-1-ol, each of the remaining five compounds was the most effective stimulus for one of the remaining receptor neurons. No obvious differences were observed in the relative proportions of receptor neurons either excited, inhibited, or unaffected between stimuli which are behavioral inhibitors and those which are behavioral synergists.

Summary

The distribution of mean spontaneous levels across all receptor neurons was positively skewed toward low levels of activity. In addition, the spontaneous activity of the A unit was usually higher than that of its companion (the B receptor neuron) within any one sensillum; however, this difference was small compared to the differences seen in the absolute levels of activity between sensilla. The responses of olfactory receptor neurons (both A and B units) to stimulation with most of the compounds studied were usually increases in the frequency of action potential production. One of the compounds, *t*-11-TDA elicited decreases in response frequency in some of the units. In general, response frequency was a linear function of the log of stimulus intensity. In absolute terms there was wide quantitative variation in the responses produced by the primary pheromone component and its geometric isomer both between and within sensilla. However, on a relative base *c*-11-TDA was often a more effective stimulus for A units, whereas, *t*-11-TDA was usually more effective for B units. The remaining behaviorally active compounds elicited responses which were considerably more variable both relatively and absolutely. No regularity in the responses of the two classes of receptors to these compounds was discerned.

DISCUSSION

An Estimate of Response Variability

The most unusual characteristic of this sample of insect olfactory receptor neurons, especially when compared to Bombykol receptor neurons, is the considerable variability in their responses to biologically active compounds. This variability is seen in responses across receptor neurons to any one compound (Figs. 4 and 5) and across compounds for any one receptor neuron (Table II). This could arise from: (*a*) strictly physical factors which somehow alter the relative accessibilities of compounds to the receptor neurons; (*b*) uncontrolled physiological factors which change the general level

of excitability of the receptor neuron system from one odor presentation to the next; and (*c*) intrinsic receptor neuron properties, such as the kind, number, and location of individual receptor sites,[3] which are differentially distributed from one receptor neuron to another. Obviously, if either of the first two factors operate extensively, then deciphering the mechanism of quality coding becomes exceedingly difficult. The third possibility, however, could provide the raw material from which a system of quality coding might be built.

Physical factors external to the sensilla, such as differences in stimulus flowrate, duration, size of the odor cartridges or their placement, can be excluded, because they remained constant both within and between compounds. However, physical relationships internal to the sensillum are not so easily controlled. Steinbrecht (1973) has demonstrated that the dendritic processes of the two olfactory receptors in a single *Bombyx* sensillum trichodeum usually have different diameters (Fig. 1 F). Consequently, they have different surface areas and different numbers of contacts between their respective dendritic membranes and the pore-tubules which traverse the thickness of the cuticle and through which odorous molecules diffuse. The diameter of these pore tubules is large (approximately 100 Å) and should not present a diffusion barrier for molecules of the sex pheromone, whose largest dimension is less than 35 Å. Assuming that dendritic membrane–stimulus molecule interactions are a necessary antecedent to action potential production, then the physical differences observed by Steinbrecht (1973) between receptor neurons within a single sensillum could give rise to absolute differences in their respective response frequencies.

The fact that there are always characteristic differences in action potential waveform and amplitude within a single RBLR sensillum suggests that there is a difference in the size of the dendritic processes of the two RBLR receptor neurons which is similar to that described in *Bombyx*. On the basis of such a difference it may be assumed that if one compound elicits higher response frequencies in one of the receptor neurons than in the other, a second compound should elicit responses that are, at the very least, in the same relative order. This hypothetical argument assumes that the receptor neurons have no differentially sensitive receptor sites of their own, but simply give a spike output that is proportional to the number of molecules interacting with their dendritic membranes.

In most cases it is impossible to obtain an independent estimate of the various contributions of these physical variables (the number of pore tubules, their distribution on a sensillum, the number of pore tubule–receptor membrane contacts/unit of membrane area and the total receptor membrane

[3] A receptor site is thought to be a molecular specialization of the olfactory receptor neuron membrane which accounts for the neurons ability to detect volatile chemical stimuli.

area/receptor cell) to the overall variability in response frequency obtained with chemical stimuli. However, instances have been observed in which the operation of these variables on the two receptor neurons of a sensillum seem equalized. For instance, if both receptor neurons of a sensillum give nearly identical responses to an intensity series of a compound then it must be assumed, in the absence of any differentially distributed intrinsic receptor membrane factors, that whatever the physical variables operating on one of the receptor neurons of a sensillum, they must be equal to those operating on the other. Assuming also, that the membrane properties of the two receptor neurons are equally nonspecific, then any other compound used as a stimulus must also elicit nearly identical response functions in the two receptor neurons. On the other hand, any deviation between the two response functions elicited by the second compound indicates that the receptor properties of the two neurons are not identical. The occurrence of such deviations clearly indicates that the two receptor neurons, contained within a single sensillum trichodeum, must have absolute differences in the kind, number, or distribution of receptor sites present on their respective dendritic membranes.

The response functions generated in the pair of receptor neurons within a single RBLR sensillum trichodeum by stimulation with an intensity series of both *c*-11-TDA and *t*-11-TDA are shown in Fig. 7. The response ratios

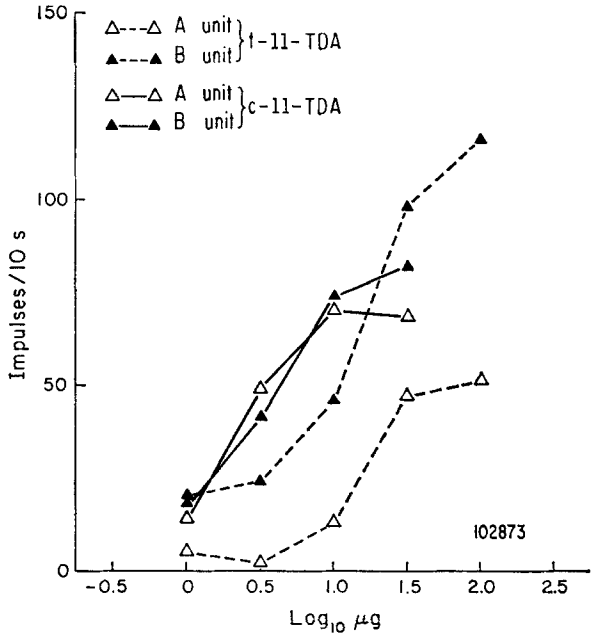

FIGURE 7. The response functions generated by the two receptor neurons of a single sensillum when it was stimulated with an intensity series of either *cis*- or *trans*-11-tetradecenyl acetate.

produced by these two receptor neurons when stimulated with the two compounds do not bear a constant relationship with one another. Specifically, both receptor neurons give nearly identical response functions when stimulated by *c*-11-TDA indicating, for purposes of the above theoretical argument, presumed equalization of all physical variables. However, the response functions elicited by *t*-11-TDA in the same two receptor neurons are quite different, especially in terms of response threshold, slope, and maxima. Consequently, these differences in response between receptor neurons cannot be simply explained by differences in physical factors which might affect the relative accessibility of molecules to the receptor neurons. Therefore, we must assume that these two receptors actually differ in some basic receptor membrane property which results in the different response functions observed.

Similar considerations indicate that this conclusion is not restricted to this one sensillum (Fig. 7) but rather applies to most of the RBLR sensilla sampled. For each of the 23 pairs of receptors examined, the difference between the response magnitude elicited in unit A and that elicited in unit B by 31.6 μg/μl of *c*-11-TDA ($[R_A - R_B]_{cis}$) did not equal the difference in response magnitude produced by the same amount of *t*-11-TDA ($[R_A - R_B]_{trans}$).

The magnitude of the deviation between these two difference scores ($[R_A - R_B]_{cis} - [R_A - R_B]_{trans}$) ranged from -41 to $+490$ impulses/10 s with a median and standard error of 88 $\pm$ 26. In the majority of the sensilla (15 out of 23), the difference scores obtained with the two compounds had opposite signs e.g. $R_A > R_B$ with *c*-11-TDA and $R_A < R_B$ with *t*-11-TDA. There is no way in which the physical factors outlined previously could affect the relative accessibilities of the two compounds such that the two receptor neurons within a single sensillum would produce response difference scores with opposite signs. Such differences in responsiveness can only arise if the two receptor neurons have different intrinsic receptor membrane properties. In the remaining sensilla (8 out of 23), the difference scores for the two compounds had the same sign ($R_A > R_B$ in 3 and $R_A < R_B$ in 5). However, even in these sensilla there were quantitative differences in the scores obtained with the two compounds which would not be expected if only physical factors were determining receptor neuron responses. Consequently, none of the differences in response observed between the two receptor neurons of a sensillum can be attributed to random or systematic differences in the physical access of stimulus molecules to the receptor neuron membrane. They can only be due to real differences in the receptor membrane properties of the receptor sites normally found on the two receptor neurons.

A second source of variability in the responses of olfactory receptor neurons to chemical stimulation could involve some uncontrolled physiological

change in the insect which alters the general level of excitability of the receptor neurons from one moment to the next. This source of variability can be estimated by comparing the variability of repeated measures of response in the two receptor neurons of a single sensillum with that obtained for the responses of the total sample of single A and B receptor neurons. If the variability of repeated measures of response in a single receptor neuron is considerably less than that observed across the total sample of receptor neurons then it is likely that the variability actually observed in the whole sample is not caused by moment-to-moment fluctuations in excitability. Therefore, the variability observed may again be attributed to differences in the intrinsic receptor site properties of the dendritic membranes of individual olfactory receptors.

The mean responses and their standard deviations as elicited by the indicated intensities of *c*-11-TDA in four individual sensilla are contrasted in Table III with the means and standard deviations observed in the sample of receptor neurons whose response functions are plotted in Fig. 4. In all cases the variability in the response of individual receptor neurons is less than 25% of that observed between receptor neurons in the whole sample. In some cases the individual receptor neuron variability is less than 10 %. As is the case with vertebrate olfactory receptor neurons (O'Connell and Mozell, 1969), the relative variability (standard deviation expressed as a percent of the mean) of the response increases as the absolute magnitude of the response decreases ($r_s = -0.90$, $P \leq 0.01$). Although there are many other sources of response variability that cannot be evaluated here, it is likely that longer term (>3 h) shifts in excitability should also be eliminated from consideration. For example, in one experiment the responses elicited in the two receptor neurons of a single sensillum by stimulation with 31.6 $\mu g/\mu l$

TABLE III

COMPARISON OF THE MEAN AND STANDARD DEVIATIONS OF RESPONSES FOR TWO INTENSITIES OF *c*-11-TDA IN FOUR INDIVIDUAL SENSILLA AND THOSE OBTAINED FROM ALL THE RESPONSE FUNCTIONS ILLUSTRATED IN FIG 4*

10.0 $\mu g/\mu l$			31.6 $\mu g/\mu l$		
Sensillum	A unit	B unit	Sensillum	B unit	A unit
1	20±4	13±6	2	80±11	48±9
3	11±3	0±2	4	99±9	75±4
all	66±31	40±25	all	79±47	54±36

* Responses in impulses/10 s rounded off to the nearest whole impulse. The listed stimuli were presented seven times to each of the indicated sensilla. The number of the sensillum indicates the order in which it was obtained from a single segment of a male RBLR antenna. The elapsed time from the first stimulus presentation on sensillum 1 to the last presentation on sensillum 4 was 3 h.

of *c*-11-TDA were 194 (in A) and 139 (in B) impulses/10 s. After 12 h of continuous monitoring this same pair of receptor neurons produced responses of 205 (A) and 150 (B) impulses/10 s for the same stimulus intensity. This represents an average increase in response frequency to this compound of less than 1 impulse/h.

From these considerations, it is apparent that both the quantitative and qualitative differences observed in the response functions of individual olfactory receptor neurons can be attributed to quantitative and qualitative differences in the intrinsic receptor site properties of the neurons and are not due to differences in their physical arrangement or alterations in their physiological state.

As in all other single receptor neuron studies, it is important to question whether the neuron sample adequately describes the properties of the receptor neuron population as a whole. In the olfactory system of insects, this can be accomplished by comparing the summated electrical activity of the entire antenna, as measured simultaneously by the EAG, with some overall estimate of single receptor neuron discharge magnitude. If these two measures agree over a range of different stimuli, it is reasonable to conclude that the sample of single receptor neurons obtained fairly represents the properties of the entire receptor population.

An estimate of the overall single receptor neuron discharge magnitude elicited by each of the compounds was obtained by computing the mean response across all receptor neurons in the sample, regardless of unit type. These means were then correlated with the magnitude of the EAG's elicited by these same compounds as reported by Roelof's and Comeau (1971 *b*). The statistically significant positive correlation between these two measures ($r_s = +0.77$, $P < 0.05$) indicates that the distribution of sensitivities observed in the single receptor neuron sample adequately represents the distribution present in the whole population of receptor neurons.[4]

Receptor Sites

As a result of their behavioral and EAG studies of male RBLR, Roelofs and Comeau (1971 *a*, *b*) proposed an "induced fit" receptor site model of insect olfaction which involved the interaction between behaviorally active compounds and receptor site protein presumably located on receptor neuron dendrites. In this model they assumed that there were four separate active regions in a single receptor site where various alignments with corresponding binding sites in different stimulus molecules could occur. These various alignments were thought to lead to conformational changes in the receptor

[4] The order obtained from the single unit studies was: *c*-11-TDA > *t*-11-TDA > 10-PDA > *c*-11-TDF > 10-UDA > DDA > TriDA > *c*-7-DDA > *c*-11-TDol; for the EAG study, *c*-11-TDA > *t*-11-TDA > *c*-11-TDF > 10-PDA > *c*-11-TDol > 10-UDA > TriDA > *c*-7-DDA > DDA.

site proteins which would in turn modulate the activity of the nerve impulse-producing mechanism of the receptor neuron. The resulting modulation of receptor neuron impulse activity would in some manner affect central nervous system function and finally lead to discriminable behavioral responses. The number of functionally different receptor sites and their relative distribution both within and between the two olfactory receptor neurons of the sensilla trichodea were unspecified.

It is clear from this single receptor neuron study that there are at least some sensilla in which both receptor neurons are capable of responding to as many as eight of the nine behaviorally active compounds used as stimuli (Table II). One explanation for these data would be that there are at least eight *functionally* distinct receptor sites/receptor neuron and that these sites may be differentially distributed between the two receptor neurons. On the other hand the number of postulated receptor sites could be reduced if quantitative differences in binding strengths between odor molecule and receptor site were allowed. Unfortunately sufficient quantitative data on all the behaviorally active compounds, are not yet available to allow us to set an upper limit on the number of different receptor sites involved. However, it is clear from the available data that the lower limit must be larger than one. For example, the data shown in Fig. 6 could not be generated by a system which has only one functionally distinct receptor site. Assume for the sake of argument that there is in fact a single receptor site for *c*-11-TDA and that there are more of them on unit A than there are on unit B. This type of quantitative distribution easily accounts for the differences observed in the two response functions for *c*-11-TDA stimulation, in that the responses elicited in unit A are always larger than those elicited simultaneously in unit B. However the responses elicited in these same two receptor neurons by *t*-11-TDA stimulation have the opposite order in that the B unit response is always larger than the A unit response. Further, the responses of the A unit at the lower stimulus intensities are inhibitions. Consequently, an adequate explanation of the opposing responses to these two compounds requires at least two different *functionally* independent receptor sites.

Quality Coding

RBLR olfactory receptor neurons are in some respects similar to those receptors found in *Bombyx* which are specialized for the detection of Bombykol. Points of similarity include: (*a*) In both insects the sex pheromone receptor neurons are anatomically segregated into the long sensilla trichodea. In addition, the receptor neurons found in the sensilla basiconica of both insects are unresponsive to these compounds. (*b*) These sensilla trichodea usually contain two olfactory receptor neurons, one of which produces a larger amplitude spike potential than the other. (*c*) The receptor neurons of both

insects are usually spontaneously active. (*d*) The olfactory receptor neuron which produces the large amplitude spike is usually more sensitive to the primary pheromone component than the receptor neuron which produces the small amplitude spike. (*e*) The receptor neurons of both insects respond to several compounds in addition to the sex pheromone.

However, in spite of these similarities, there are a number of differences, several of which implicate the existence of profound differences in the mechanism of quality coding utilized by the two species. These differences include: (*a*) Inhibitory responses can be observed in RBLR receptor neurons especially with *t*-11-TDA stimulation. The absolute spike frequency of these inhibitory responses is usually much less than the spike frequency of excitatory responses and has as its upper bound the level of spontaneous activity for each neuron (mean, 28 impulses/10 s). The fact that inhibitory responses are rarely seen in *Bombyx* receptor neurons may simply be a reflection of their low levels of spontaneous activity (mean, 1 impulse/10 s). (*b*) The range of compounds which elicit responses in *Bombyx* (i.e., the response spectrum) is constant from one Bombykol receptor neuron to another (Kaissling, 1971). In contrast, the response spectrum of RBLR receptor neurons varies from one neuron to the next (Table II). In addition to this qualitative variability, there is considerable quantitative variability in response across receptor neurons for any one compound (Figs. 4 and 5). Such quantitative differences in the response of Bombykol receptor neurons have not been noted. (*c*) Any compound which elicits spike activity in the Bombykol receptor neuron (above a certain threshold level of response) will also give rise to the same behavioral response as normally elicited by Bombykol when presented to the whole animal at an equivalent intensity (Priesner, 1969). The threshold for this behavioral response is quite low, requiring only a single extra spike per second in less than 1% of the available receptor neurons irrespective of the compound used to effect such an increase (Kaissling, 1971). This is not the case in the RBLR. Most of the compounds used in this study elicited strong excitatory responses in the trichodeal receptor neurons. However, these compounds do not elicit identical behavioral responses when presented to the whole animal. In particular *c*-11-TDA elicits one set of behavioral responses whereas *t*-11-TDA elicits another, yet both compounds excite the same set of trichodeal receptor neurons.

It must be concluded from these data that a labeled line system of quality coding, at least for the primary pheromone component and its geometric isomer, is not operating in the RBLR moth. If it were I would have expected to find at least two different classes of trichodeal receptor neurons each with its own unique and nonoverlapping set of chemical specificities. From the laboratory bioassays one could have expected a receptor neuron exclusively sensitive to the primary component (*c*-11-TDA), and a second ex-

clusively sensitive to the secondary component (*t*-11-TDA). The observed data bear little relationship to these expectations. In fact all of the compounds used as stimuli modulated the spike activity of many of the same receptor neurons. In no case could one deduce a cause-effect relationship between the absolute amounts of spike activity elicited in a particular receptor neuron and the behavior elicited by the same stimulus compound. Therefore, the encoding of odor quality for the sex pheromone system and its modulators cannot be a labeled line process in RBLR.

It should also be apparent from these data that there are a range of stimulus intensities within which two (or more) different compounds can elicit the same absolute discharge magnitude in a given receptor neuron. For example, both 3.16 $\mu g/\mu l$ of *c*-11-TDA and 100 $\mu g/\mu l$ of *t*-11-TDA produced responses of 50 impulses/10 s in the A unit illustrated in Fig. 7. Obviously the absolute discharge magnitude in a single receptor cannot be used to provide a unique code for odor quality in the RBLR moth.

Although we can clearly rule out two possible quality-coding schemes (*a*) the simple presence or absence of activity in a particular receptor neuron (a labeled line), and (*b*) the absolute magnitude of any such discharge, there still remain a large number of codes which may fit the available data.

Chief among these is the "across-fiber pattern theory" proposed to account for quality coding in vertebrate olfactory receptor neurons (O'Connell and Mozell, 1969). In this scheme, odor quality is encoded by the *relative* amounts of spike activity elicited in an ensemble of receptor neurons. Each discriminable compound is thought to elicit a unique pattern of activity across the entire ensemble of receptor neurons. This contrasts sharply with the labeled line system where each discriminable compound elicits activity in a unique and nonoverlapping segment of the available receptor neurons. That such relative differences in response exist in the RBLR data can be seen most easily in Fig. 6. At each of the intensities tested, *c*-11-TDA elicited larger responses in the average A unit relative to the responses elicited in the average B unit. The opposite relation held for *t*-11-TDA; the B unit produced larger responses than did the A unit. This relationship was also a consistent observation in the sample of single receptor neurons. Therefore a simple comparison of the response ratios elicited in the two receptor neurons of a sensillum would unambiguously encode odor quality, at least for *cis*- and *trans*-11-tetradecenyl acetate.

The distribution of sensitivities seen in these same receptor neurons when other compounds are used as stimuli (Table II) is considerably more complex. This additional complexity could of course serve to increase the fine structure of the across-fiber pattern. However, we do not know whether these differences in sensitivity will be constant in the face of changes in stimulus intensity. This latter stipulation must be a requirement for an adequate

across-fiber pattern code in order to avoid confounding odor quality with odor quantity.

Finally, it must be noted that codes involving many other aspects of receptor neuron discharge may also be likely candidates. For instance, there are often clear-cut differences in response latency, duration, and interspike interval distribution between A and B units for a given compound (Fig. 3). If there are characteristic differences in these variables for different chemicals, then any of these temporal characteristics of receptor neuron discharge could also code for odor quality.

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