

## Temperature-dependent Dry Receptor on Antenna of *Periplaneta*. Tonic Response

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**Summary.** Identified as a dry receptive unit by transitory impulse frequencies up to about 200 imp/sec during rapid drops in humidity (Fig. 3) and which no other modality elicited, the dry unit on the antenna of *Periplaneta americana* is characterized by a regular (Fig. 4) and relatively high stationary impulse frequency (10–65 imp/s). Without exception the stationary discharge rate (tonic frequency) rose with falling values of stationary absolute humidity at constant temperature, and with rising values of stationary temperature of ambient air at constant humidity. Enthalpy and evaporation cooling appear to be ruled out as exclusive adequate explanations for this double dependence. No matter whether tonic frequency is plotted against absolute humidity based on either volume of moist air or weight of dry air, or against partial pressure of water vapor in ambient air, or the difference between saturation and partial vapor pressure, or against relative humidity, the dependence on the temperature is not eliminated (Figs. 5–7). Because a temperature range of about 20 °C and all humidities between 0 and 100% occupy the same large segment of the unit's tonic frequency spectrum (Fig. 8), the unit is termed bimodal.

### Introduction

The existence of hygroreceptive sensilla on the antenna of *Periplaneta americana* as previously described (Altner et al., 1973) has been confirmed (Yokohari et al., 1976). These authors too found a dry and a moist receptive unit combined there in a single sensillum with a cold cell. The moist (M) receptive cell responds to increasing humidity with rising impulse frequency and the dry (D) receptive cell with diminishing frequency. The opposite occurs when the two types are stimulated with falling humidity. Hygroreceptors antagonistic either in type (Madge, 1965) or in reaction (Begg and Hogben, 1946) had been the subject of conjecture in order to explain the reversal of humidity preference in choice chambers after amputation of body parts in various arthropods (for list and

critical discussion, see Syrjämäki, 1962). Though their relationship to behavior as antagonists is by no means clear, the existence of an antagonistic pair was demonstrated electrophysiologically in *Locusta* by Waldow (1970) and shown to be present in the recordings of an M-unit on the antenna of *Apis* by Lacher (1964).

Waldow often found (1970) the antagonistic pair in combination with a cold (C) receptor in the same sensillum. Bernard (1974) has also encountered this triad, 1 M, 1 D, and 1 C cell in single sensilla on the antenna of the predacious bug *Triatoma*. The cold cell is absent on the innervated maxillary and mandibular tips (= stylets) of the beak of both *Triatoma* (Bernard, 1974; Bernard et al., 1970) and *Rhodnius* (Pinet, 1970). But there are again two types of receptors present which respond antagonistically to humidity. They also respond antagonistically to minute tugging and pushing against the stylets. These are of special interest because of the possible general course they indicate which primary processes might take. Do hygroreceptors react to humidity because their shape, or extent of deformation, is governed by the amount of moisture in the air? Results here rule out this possibility only as an adequate and exclusive explanation, unless deformation should depend on temperature too.

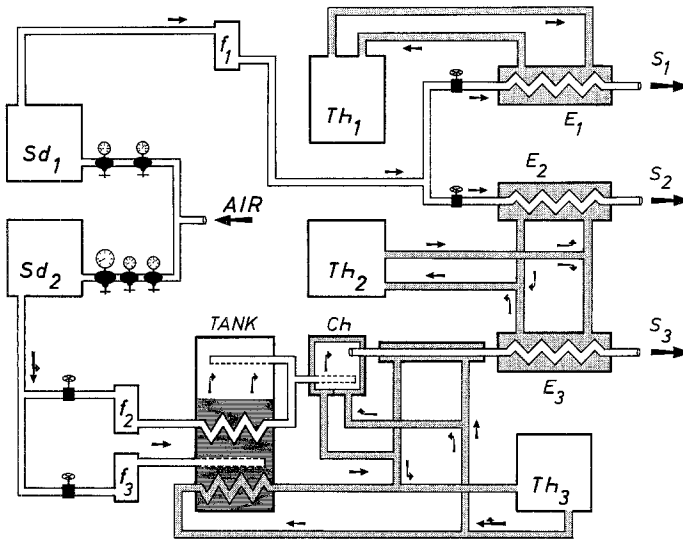
The present paper is concerned only with *Periplaneta americana's* antennal dry receptor. Treatment is limited to identification, occurrence and tonic response.

## Materials and Methods

*Stimulus.* Three air streams emerging from jets 7 mm in diameter at 2.6 m/s and laminar for more than 6 cm provided the medium for both moisture and temperature stimuli (Fig. 1). The air was cleaned and dried in two separate units by passing it through concentrated sulfuric acid and then over KOH pellets to remove acid vapors. Air from one such unit was used as zero moisture stimulus. Air from the other was divided into two substreams. The first was bubbled out through hundreds of small holes in polyethylene tubing firmly anchored for its entire length in a tank of ion-exchange water of constant depth and temperature (46.6 °C). The second substream passed through a heat exchanger in the same tank but remained dry. The two were then united. By regulating the flow of each (Rota-meters) their total flow could be held constant and their proportions varied. Thus the moisture of the air emerging from the jets could be set at different levels without altering its velocity.

In order to guarantee homogeneity in the mixture, the air was driven through a 1.4-liter series-connected vessel at the same 46.6 °C after uniting the two substreams. The advantage of the vessel was to smooth out small random fluctuations in moisture content which might conceivably develop and follow each other down through the heat exchangers and out the jets if the mixing were done simply at the "Y" junction of the tubes carrying the moist and the dry air.

The vessel had the further effect, however, of increasing the time needed for the air at the jets to assume a final humidity value after setting the apparatus for the new level. For the cross-section areas of the openings in and out of the vessel were about 200 times smaller than that of the vessel itself. Assuming a flushing efficiency of only 20% (20% of initial gas replaced by new with passage of a volume of gas through the vessel equal to its volume), 21 flushings would be needed to reduce the percentage of the initial gas in the vessel down below 1%. This error was considered tolerable even though its direction would tend to make the difference between two end values smaller than is actually the case whenever the difference is obtained from a successive pair. Such an error is important, for the difference forms the denominator of the ratio: difference in impulse frequency to corresponding difference in moisture level. Too low a value in the denominator would tend to produce an unjustifiably high value for the ratio, here the expression for sensitivity of the receptor to the level of moisture. At a flow rate of 750 l/h used, the 1% level of error

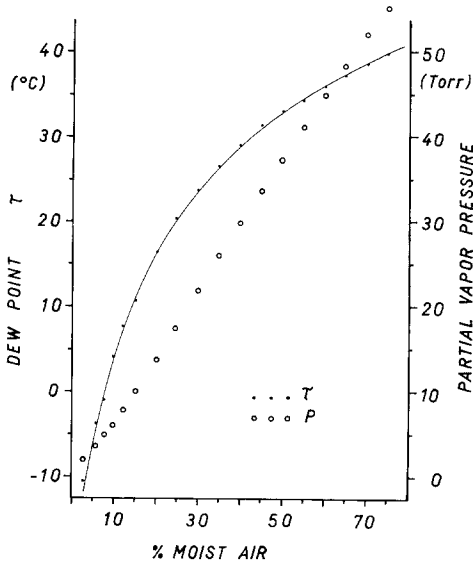


**Fig. 1.** Flow diagram illustrating production of 3 laminar air streams, two ( $S_{1,2}$ ) at partial pressure of water vapor approaching zero in this simpler case, and two ( $S_{2,3}$ ) at same temperature. Thermostats (COLORA N and KT-30) at  $Th_{1,2,3}$ . Vapor pressure predetermined by bubbling air through TANK of water at constant depth and temperature (46.6 °C) and mixing with dry air in proportions regulated by flow meters ( $f_{2,3}$ ; ROTAmeters). Homogeneity of mixture enhanced by 1.4 liter series-connected chamber ( $Ch$ ). Only water-jacket insulation shown.  $Sd_{1,2}$ : scrubbing and drying units preceded by batteries of reduction valves to eliminate significant fluctuations in initial pressure. By sending moist air through heat exchangers at  $E_1$  and  $E_2$ , and dry air at  $E_3$ , an arrangement often utilized but not illustrated here, rapid shifts in temperature can be made at a common but variable vapor pressure

would be reached in somewhat under 2 min. Less than 4 min would be needed at half the flow rate. This time was the minimum permitted to elapse between recordings.

The temperature of the stimulus air was then controlled by a further set of heat exchangers. As a result two of the streams had the same partial pressure of water vapor at different temperatures, and two had the same temperature at different vapor pressures. It should be noted that the effect of imposing a temperature on the air streams other than the original 46.6 °C is to change both their relative and absolute humidity so long as the streams contain any moisture at all. For under the supposition that the rate at which moisture is picked up by dry air flowing at a constant speed through a column of water at constant temperature and depth is constant, the absolute humidity at this point is also constant and so is the vapor pressure. The total pressure is about 0.65 Torr above that of the surrounding atmosphere and remains so whatever temperature in the 17° to 37 °C range is imparted by the heat exchangers. But in this open-ended system it is the pressure of the surrounding atmosphere rather than the temperature of the heat exchangers which determines the pressure of the gas emerging from the jets. The work done by the heat exchangers here does not affect pressure but volume, and with volume density. In such a case the absolute humidity, defined as the weight of water vapor per unit volume of moist air ( $g/m^3$ ), also varies with absolute temperature. When the temperature falls from 37 to 17 °C, the density of the mixture at the jets rises and with density also absolute humidity by about 7%.

Unfortunately absolute humidity does not enjoy uniform definition. The one used here agrees with most works consulted, including the Handbook of Physics and Chemistry (1969). The Lexikon der Physik (1969) offers not only volume but also mass of air as a reference parameter. Others such as Frauenfelder and Huber (1966) perhaps more reasonably define it simply as the partial pressure of water vapor.



**Fig. 2.** Calibration of flow ratio of moist to dry air (expressed as percent moist air in mixture) for partial vapor pressure and dew point as determined from difference between wet and dry bulb temperature readings. Sequence of readings rather random. In this open-ended system, absolute humidity is dependent on temperature superimposed by heat exchangers but dewpoint and vapor pressure are not. Values at jets reproducible within 3%. Ordinate scaling factors in relation to each other as well as the relative position of their zero values are arbitrary

That relative humidity varies with temperature follows immediately from its definition as the ratio of the vapor pressure at a given temperature to what it would be at saturation for the same temperature. Because the vapor pressure at the jets remains constant and saturation pressure increases with temperature, relative humidity also falls as the temperature rises.

Summarizing important aspects, once a mixing proportion of moist to dry air is set, partial vapor pressure remains constant at the jets at all temperatures above the dew point, but each change in temperature produces a change in both relative and absolute humidity, both in the same direction (see Fig. 2 for calibration). Such calibration dispensed with the difficulty of humidity measurements within the air streams during experiments.

The 3 jets were moveable and could be directed individually at the same small section of antenna mounted on a prism edge about 15 mm away. Switching was done by electromagnets. As each jet was snapped into position, attached gates deflected but did not interrupt the streams of the other two. Since the humidity and temperature of the streams were already at terminal values when switching occurred, the air surrounding the antenna assumed transitional values only during the few milliseconds of actual switching. No attempt was made to measure these transients.

*Temperature* was measured within the air stream by means of a small uncoated bead thermistor (Fenwall Electronics, BC 32 L1), located about 3 mm behind the recording electrode.

*Recording.* Impulses were picked up extracellularly between tungsten electrodes, one inserted a few millimeters into the antenna through the tip and the other at the sensillum base. Recording was done photographically from a Tektronix 502 A oscilloscope screen with a Tönnies camera.

*Evaluation.* Mean frequencies are the per-second impulse interval count (to the nearest 0.1 interval) for fixed periods of time.

*Definition.* The letter H is reserved in this paper for humidity and M for moisture. Enthalpy, normally symbolized by H, is not abbreviated here.

## Results

### *Identification*

The dry (D) receptive unit of the antenna of adult *Periplaneta americana* males was identified by its response to sudden changes in humidity (H). When H was quickly lowered by shifting from a moist to a dry air stream of the same temperature, the impulse frequency of D rose to heights elicited in no other stimulus situation (Fig. 3). Neither sudden changes in temperature, nor gusts of air up to 10 or 20 m/sec, nor variations in light intensity, nor magnetic field, nor stimulation with some 100 dry air-borne odors produced such reactions. The assortment included amines, esters and homologous series of short-chained alcohols, aldehydes and acids to which *Periplaneta* is known to respond (Sass, 1976). Nor did odors from fresh fruit, meat and cheese affect frequency other than as would be predictable from the moisture content of these foods. The only modalities with a clear effect on the firing rate were H and temperature (T), and of these only H elicited such responses as are illustrated in Figure 3. The reaction to rapid T-change is less pronounced. Stimulation by direct contact with substances in or out of aqueous solution was not undertaken, since the sensilla in which D-units were found, were protected from direct contact with the substrate by the long bristles which surround them. A systematic attempt at direct mechanical deformation of the sensillum during recordings was not undertaken either, although many D-units withstood variation in lateral pressure exerted by the electrode in the sensillum base. In such instances small movement appeared to have little effect. Easily reversible F-changes corresponding to movement of the electrode were not observed. But the H-reactions were reproducible at will, though with variation depending on temperature and time allowed for recovery after previous stimulation.

Upon reverse stimulation (a rapid shift from dry to moist air) the firing rate dropped at once to frequencies much lower than for any steady values of H or T studied. These reactions to sudden changes in H are the exact opposite of those of the M-unit as described by Lacher for *Apis* (1964), Waldow for *Locusta* (1970), Bernard for *Triatoma* (1974), and which often occurs too in the same sensillum with the D-unit in *Periplaneta*.

### *Occurrence*

*Location.* Just as with the antennal cold receptor (C) of *Periplaneta americana*, receptors for dry air (D) and moist air (M) were found in the neighborhood of the membranous intersegmental joints towards the distal end of the segments and most commonly on the distal half on the antenna. Sometimes more than one hygroreceptive sensillum per segment was detected, but there were many segments on which none were found. Only the area about 120° to either side of the ventral midline of the antennae of adult males was examined.

*Combination.* In the case of *Locusta migratoria*, Waldow (1970) considered it quite probable that C-, D-, and M-receptors normally occur as a triad in single

**Table 1.** Combinations in which D, M, or C receptors were found alone or in combination in single sensilla and frequency of these combinations in 301 sensilla for which records of combinations were kept

Receptors actually found in sensillum. Only	Frequency of occurrence	Occurrence as percent of 301 sensilla	Would correspond to failure to contact receptor:	Total frequency in all combinations	Total percentage in all combinations
D	9	3%	(M+C)	D = 54	18%
M	25	8%	(D+C)	M = 155	51%
C	130	43%	(D+M)	C = 247	82%
M+D	20	7%	(C)		
C+D	7	2%	(M)		
C+M	92	31%	(D)		
C+M+D	18	6%	—		
Total	301	100%			

sensilla. The situation on the honeybee antenna is susceptible of the same interpretation (Lacher, 1964; Waldow, 1970). Although it was not the purpose of this study to determine the relative frequency of occurrence of these three receptors with respect to one another, a tally was kept for the combinations encountered in 301 sensilla, roughly 20% of those monitored.

This tally is offered in Table 1. As such it admits of at least three interpretations, none of which can be rigorously excluded on the basis of the tally alone (see Discussion). At yet there is no clear evidence here for another unit than C in combination with D and M. In addition to units of uncertain modality there have been hints of two M-cells in a single sensillum or even of a warm unit responding to a rapid rise in T with a marked increase in F, but too short-lived for critical testing.

#### *Extracellular Impulse Polarity and Amplitude*

The form of D-receptor impulses displayed a high degree of variability. Not only did their amplitude differ from one recording to another and often tend to diminish with time during single recordings, but the ratio of their amplitude to that of the moist (M) and cold (C) receptive cells was not constant either. The amplitude of the dry receptor on the locust and honeybee antennae tended to the lowest of the three. But *Periplaneta*'s was by no means always the lowest. This variation was further compounded by the influence of temperature and humidity. Depending on the stimulus situation, the amplitude changed both absolutely and relatively to that of C- and M-impulses. These changes were reversible within the limits placed by the not infrequent general diminution with time. Even their polarity was affected. As a rule the impulses were positive with a slight negative phase small to the point of disappearance in the base line. But especially during rapid changes in the stimulus situation, the positive

phase could diminish and the negative rise and make the impulses clearly bipolar. Nor did these changes in amplitude necessarily proceed at the same rate. The positive phase often became quite small before the other began to grow obviously longer. Thus the total absolute amplitude of the two phases was not constant either.

The cause of these changes in form is obscure. The shape of the electrode, its depth and its position relative to the three receptors surely differed to some extent with every insertion. On the basis of the methods employed one can do little more than speculate concerning impedance transients or ion accumulation in individual structures separating the excitable membrane from the electrode surface, or concerning the degree to which they reflect primary processes.

But if the cause is not clear, the effects were quite important. The changes in the amplitude and especially in the ratio of amplitudes of the D-cell to those of the C- and M-cells repeatedly ruled out discrimination by amplitude alone. Impulse form was often decisive and had to be determined visually from impulse to impulse under considerable magnification. Even then identification at constant levels of M or T was sometimes possible only because the sensillum had been subjected to a fast and short (0.2 s) H-shift towards the end of individual recordings. The resulting dramatic change in frequency identified the impulses and an examination of their form at the onset of the F-transient characterized those impulses preceding it.

### *Tonic Response*

*Double Dependence.* A cursory inspection of Figures 3 and 4 shows that the D-receptor continues to discharge as the speed with which H and T change approaches zero. The question immediately suggested is whether the discharge rate, F, is a function of steady states of either or both of these parameters. From the same figures it is also clear that F is dramatically influenced by sudden variation in vapor pressure. Thus an answer to the above question would necessitate considerable care that levels of H had actually been reached before recording. To this end 7 to 10 min were allowed to pass after setting an H-level, just as was done in calibrating the settings themselves for humidity (see Fig. 2). In this length of time the difference between wet and dry bulb temperature measurements no longer changed perceptibly. The time also gave the receptor a chance to adapt to the H-level.

Since H-levels could be established more quickly than T-levels, the former were varied while T was held constant. In the interests of saving time temperature changing at the rate of a few tenths of a degree per hour during experiments was sometimes deemed tolerable, since rates of T change 30 or more times greater had no discernible effect on F (Loftus, in prep.). Then after measurements at 5 to 8 H-values in random sequence, the T-level was altered and the responses to a similar set of H-levels recorded. No more than 3 repetitions of this procedure were successful for any single receptor. As an approximation of the resulting characteristic humidity-response curves, the linear regression for each set of responses to the varying levels of H was determined by the method of least

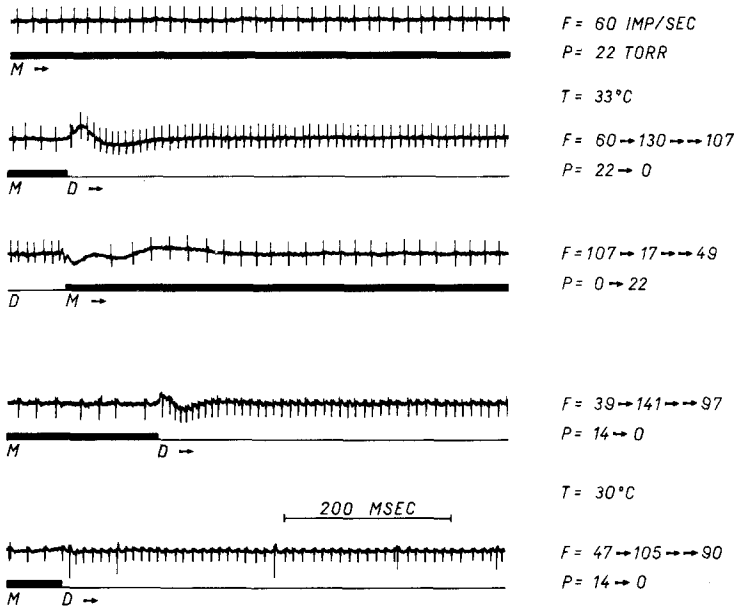


Fig. 3. Characteristic D-receptor response from single units in 3 animals (lines 1-3, 4, 5). Note variation in impulse form. M, moist air stream. D, dry air stream. F, impulse frequency. P, partial pressure of water vapor.  $\rightarrow$ , sudden jump or drop in F or P.  $\rightarrow\rightarrow$ , gradual change in F from maximum or minimum to average value for about the first 0.8 s after shifting between air streams. P-values at 33 and 30 °C correspond to 58, 44 and 0% relative humidity

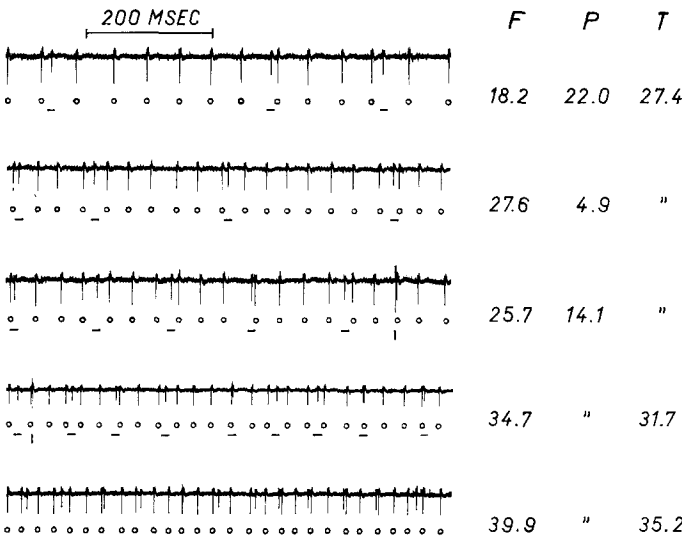
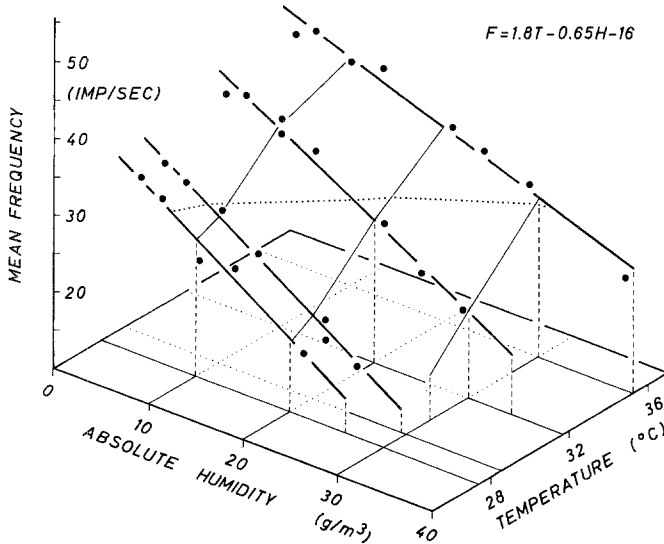


Fig. 4. Five tonic responses (F=imp/s) from single D unit (o o o) at combinations of partial pressure of water vapor in air and temperature as indicated (P=Torr, T=°C). The tendency of the F level to be higher at lower values of P and higher values of T is general. (---) Unit of uncertain modality. (III) Cold unit





**Fig. 5.** Tonic response of single receptor to constant absolute humidity ( $H_a$ ) and constant temperature ( $T$ ). Each point is the average frequency ( $F$ ) (i.e. reciprocal of mean interval between impulses) for four sec out of a single response to a given combination of  $H_a$  and  $T$  values. Heavy straight lines, whose projections on base plane are parallel to  $H_a$ -axis, are linear regressions determined from least squared deviations of all  $F$  and  $H_a$  combinations at any one  $T$ . Dotted diagonal is isometric: indicates the set of  $T$  and  $H_a$  values evoking 28 imp/s. Equation is that of regression plane calculated (but not shown) also by method of least square deviations. Because the numerical values of  $H_a$ , water vapor content ( $\text{g H}_2\text{O}/\text{kg dry air}$ ), and vapor pressure in Torr are so close to each other, Figure 5 can represent in principle the plots of  $F$  against  $T$  and each of the 3 moisture parameters

squares for each  $T$ . These regressions in turn also indicate the dependence of the response on  $T$ . By connecting the curves at successive constant humidity points, approximations of temperature-response curves emerge for different constant values of  $H$ . For this double dependence see Figure 5. The  $T$ -values shown are of course averages for the actual values measured whenever  $T$  was slowly drifting. For this reason the individual measured values and not average values of  $T$  were used in determining the equation at the upper left in Figure 5 as well as in all other figures where a double regression is indicated.

*Sensitivity.* The steady discharge rate rises at all temperatures investigated as the level of absolute humidity diminishes (Fig. 5). Closer inspection of individual points however, indicates that the humidity-response curves deviate from linearity. They appear steepest where  $H$  is greater and  $F$  correspondingly low. But as the  $F$  level is driven upwards by decreasing  $H$ , the slope tends to diminish. Thus a given difference in moisture level produces a greater difference in  $F$ , both absolutely and even more so relatively, in moist air than in dry air.

Concretely, at  $35.5^\circ\text{C}$  the gain fell off by a factor close to 4, from about  $0.8 \text{ (imp/s)/(g H}_2\text{O/m}^3)$  in moist air to about 0.2 in dry. Where the information flow therefore could be greatest by reason of higher impulse frequency, it appears to be lower because  $F$  is modulated both absolutely and relatively more at

lower than at higher values. Unless reliability of response increases considerably as  $F$  rises, resolution will also be greater at the lower end of the  $F$  spectrum. The number of successful recordings however is still too small to provide a direct and reliable estimate of resolving power.

Figure 5 shows with equal clarity a sensitivity to temperature level.  $F$  rises with the rising level of  $T$  at all values of  $H$  examined. No exception to this tendency was found in any receptor between 20 and 38 °C. At some higher temperature impulse frequency will begin to fall off again, but these temperatures were not investigated. From this receptor alone it is difficult to state quantitatively the relationship between the  $H$ -level and the slopes of the curves,  $F=f(T)$ . In general though, it does appear that the slope, and therefore the sensitivity for which it is the expression, is greatest where moisture is greatest. The reactions of other D-receptors support this contention. Thus not only is sensitivity to the level of moisture greater where moisture is greater. Sensitivity to temperature also appears to be so. Summarizing, where frequency is lower, frequency gain is greater, i.e. where moisture is higher or temperature lower or both. It should be borne in mind however that the causes for diminishing sensitivity at lower values of  $M$  and higher  $T$  are probably much more complex than the relationship exhibited in Figure 5. Intrinsic limitations on the receptor's ability to maintain a high discharge rate or to increase it may influence  $F$  at its higher values as much or more than  $T$  or  $H$  themselves do.

## Discussion

### *Occurrence*

Table 1 admits of at least three interpretations. The first possibility is that the distribution of combinations of C-, M-, and D-units actually encountered in single sensilla approximates the real distribution and that triads are the exception, not the rule. The second is the exact opposite: triads are the rule and the failure to contact one or the other of three units results from improper electrode positioning or even damage done through its insertion into the sensillum base.

The third is in between: only one or two of the three normally occur in triads; the other(s) may occur independently. This idea receives support, but by no means proof, from the fact that C-units found in combination with M- or D-cells seemed often, though not always, slower and less sensitive than those previously investigated (Loftus, 1968). Moreover, fast C-units were sometimes found on the same segment, but not in combination with M- and D-cells. It was not uncommon for sensilla with fast C-cells to lie in a column involving several segments of the antenna and for the D- and M-cells to lie in another about 60° to one side. More important, Sass has found C-units repeatedly in combination with meat odor receptive cells in sensilla where M- and D-units are consistently absent (Sass, 1976; Altner et al., 1976). The tally bears out this result. C was encountered alone almost as often as all other possible combinations of the three taken together. But what about D and M? That each

occurred about five times more often in combination as alone speaks for their being bound to triads. That M was found about three times as often as D is compatible with this idea if one further assumes D to be more vulnerable and/or M better situated in the sensillum for recording. The assumption however is only an assumption, suggested by an hypothesis and not yet borne out by unambiguous experiment. The ratios indicated by the tally would not be contradicted if neither D nor M, or only one of the two, or both were bound to triads. Regarding C, however, the findings of Sass coupled with the tally make a very strong case for the contention that C may occur independently of D and M.

### *Adequate Stimulus*

Even though there is a clear dependence of the dry receptor's firing rate on levels of absolute humidity and temperature of the surrounding air, still one cannot simply infer from this dependence that these two parameters represent the adequate stimulus. In itself absolute humidity is the measure of both density and concentration; that is, the number of grams of water vapor in a cubic meter of moist air. But if instead of the volume of moist air the mass of dry air is taken as the basis of concentration (g water vapor/kg dry air = water vapor content), the concentration becomes independent of T in this open-ended system where  $H_a$  can vary as much as 7% between 17 and 37 °C. For at constant atmospheric pressure the heat exchangers alter the volume and density both of the air streaming through them and of its component gases by the same factor at a given change in T. Thus the weight-to-volume ratio as in  $H_a$  changes with T but not the weight-to-weight ratio as in water vapor content (see Methods). Another alternative perhaps even more suitable would be partial pressure of water vapor ( $P_w$ ) since it is independent of both T and volume under isobaric conditions. If water molecules are conceived as influencing the response by entering or leaving the sensillum,  $P_w$  would have the added advantage of containing force in its definition. But plots of F against T and either  $P_w$  or water vapor content resemble Figure 5 quite closely largely because at a given dew point and T the numerical values of  $H_a$ ,  $P_w$ , and water vapor content are very close to one another in the domains investigated. Thus in these three cases the double dependence of F on T and airborne moisture is maintained. Nevertheless the possibility is still not excluded that the double dependence is not necessary and would disappear with the proper choice of parameters involving T and humidity.

Such parameters would fall into two groups: one on the side of heat and the other on the side of water vapor. Apart from mere formality, each alternative would have considerable consequences for primary processes. In the first instance water molecules would affect the response by influencing heat transfer between the sensillum and the air moving past and might themselves neither enter nor leave the sensillum. In the second water molecules would enter or leave the structure and might thereby change its shape, as appears to be the case with the maxillary stylets of predatory bugs (Pinet, 1970; Pinet

and Bernard, 1972; Bernard, 1974), or perhaps the electrolyte concentration within a critical substructure of the sensillum (cf. Pielou, 1940).

*Enthalpy.* In their treatment of moisture receptive (M) units in the maxillary stylets of *Triatoma* and *Rhodnius*, Bernard and Pinet suggested in the works just mentioned the enthalpy or heat content of stimulating air as a single unifying parameter. This choice would seem reasonable. For of the five state functions involving the first and second laws of thermodynamics, enthalpy is the most suitable for dealing with isobaric processes (Brdička, 1958; Frauenfelder and Huber, 1966). Even during experiments running for several hours variations in atmospheric pressure hardly exceed 1%. Under such conditions a change in enthalpy represents the amount of heat a given mass of moist air yields up or absorbs as its temperature is changed through a given range. The change in enthalpy is determined by the heat capacity of the gas mixture, by the extent of the temperature range, and by phase changes encountered within the T-range.

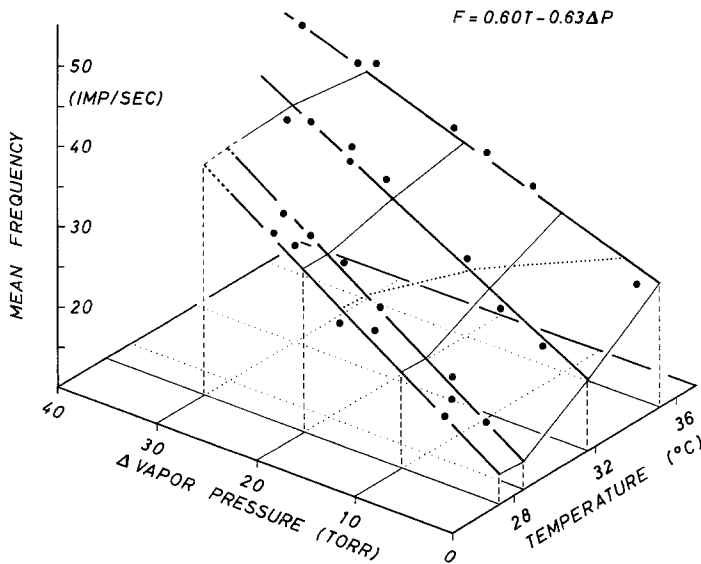
As heat flows into a given mass of air and its temperature rises, its heat content goes up. Further, as water vapor is added, enthalpy goes up again and for two reasons. First, the heat capacity of pure water vapor is about 15% higher than that of dry air under isobaric conditions. The effect is not great because in the 17 to 37 °C range no more than 6.5% of the volume of moist air can be water vapor without exceeding saturation vapor pressure (see tables in D'Ans-Lax, 1967). Nevertheless, as the proportion of water vapor in the mixture goes up, so does heat capacity, and with heat capacity, the amount of heat which must flow into the mixture to raise T by a given amount. Second, heat is also needed to evaporate water and bring it into the system. Though T of water actually evaporating does not change, heat must flow into it for evaporation to take place. Thus enthalpy rises on all three counts, as can be seen in the reverse process. The higher T, the more heat a given mass of air can yield before it has cooled to any fixed end value. The greater H, the more heat the mixture can yield for each degree it is cooled; and the greater H, the more heat can be given up in condensation. But because enthalpy rises with both T and H, enthalpy alone cannot account for the tonic response of the D-unit. For under the supposition that spiking frequency is determined by enthalpy alone and that it rises simply because enthalpy rises, then frequency must rise whenever enthalpy does. But such is not the case. In the D-unit frequency increases with rising temperature level. But it falls with rising H, although enthalpy rises under both conditions. In a moisture (M) unit, such as on *Rhodnius* or *Triatoma*, the effects of rising T and H could be interpreted as augmenting each other simply on the basis of their common increasing enthalpy. But they can't cancel each other out here on the same basis. Effects do not differ because of what their causes have in common.

Nevertheless it should be pointed out that there is some question as to the extent to which thermodynamic state functions are even applicable to tonic responses to T and H. In order to determine enthalpy under isobaric conditions, one varies T at constant pressure and measures calorimetrically the amount of heat a given mass of gas gives off or absorbs as T moves through a definite

range. But during tonic responses the only T-value of significance is the single temperature at which the response occurs. For the response persisted for indefinite periods and so far as could be determined was the same whether given T- and H-levels had been approached from above or below. But even if one would concede the receptor might have access to enthalpy in some absolute sense even at constant T, the above argumentation applies equally well. Whatever its reference value, enthalpy rises as both T and H rise. But impulse frequency rises only with the level of T. It falls with the level of H. Thus the conclusion is the same in all cases. Whether thermodynamic state functions are applicable to the experimental situation or not, in neither case does enthalpy explain the response.

*Evaporation Cooling.* Another possibility might be evaporation cooling (cf. Pielou, 1940). Of course evaporation would have to be continual to explain the persisting levels of F which depend on levels of H and  $T_a$ , the temperature of ambient air. But one might envision a continual though slight discharge of aqueous solution through interruptions in the cuticular wall of the sensillum (structure under current investigation: Altner et al., 1976; Yokohari et al., 1976) to account for continual cooling, which would in turn vary with humidity in much the same way as a psychrometer. This consideration however is easily eliminated. When the receptor is stimulated at different  $T_a$  by air with H close to saturation, little evaporation could occur. If anything, a layer of moisture might even tend to collect on the surface of the sensillum. In this situation the difference between  $T_s$  (the temperature of the sensillum) and  $T_a$  would be close to zero. Here it becomes clear that steady F is higher when the level of  $T_s$  is higher. For F rises with  $T_a$ , and where little evaporation could occur,  $T_s$  must rise with  $T_a$  too, and therefore F with  $T_s$  as well. But at a given  $T_a$ , the drier the air (i.e., the smaller H is), the more the evaporation. Evaporation would cool the surface of the solution. Heat would flow out of the sensillum and  $T_s$  would go down. Therefore F should also go down. But the facts contradict this conclusion. F rises as H goes down.

*Metabolic Warming.* A further alternative might be the flow of heat ( $dQ/dt = \text{joules/sec}$ ). As with evaporation cooling, in order to explain tonic responses, the flow would have to be maintained while H and  $T_a$  are held constant. Since the sufficient and necessary condition for thermal equilibrium, however, is equality of temperature, heat flow could persist at constant H and  $T_a$  only when  $T_s \neq T_a$ . With evaporation cooling ruled out, one might envision a metabolic heater within the sensillum to hold  $\Delta T = T_a - T_s < 0$ . (The negative value for  $\Delta T$  is chosen to indicate outward flow of heat.) To account for the response to  $T_a$  and H, heat flow would also have to vary with the levels of these two. Such a consideration seems quite unlikely, though. The speed of the air (2.6 m/s) crossing the antenna is very high relative to the diameter of the sensillum or even to that of the antenna: about  $2 \times 10^6$  sensillum diameters or roughly  $10^4$  antenna diameters per second. Relative to the sensillum the mass of air contacting it per second is presumably also very great. Thus it becomes very difficult to imagine how  $\Delta T < 0$  could be not only maintained by the sensillum



**Fig. 6.** Tonic responses of same single receptor as in Figure 5 as function of  $T$  and of the difference between saturation vapor pressure and partial vapor pressure of the surrounding air. The greater this difference, the drier the air; thus the reversal of the moisture axis. Construction as in Figure 5. To facilitate drawing, regressions were extended as dashed lines into area which would suppose partial pressure of water vapor beyond saturation. Heavy dotted diagonal: equal frequency curve

but even modulated by constant values of  $H$  and  $T_a$ , and in doing so produce differences in  $dQ/dt$  sufficient to govern  $F$ . A detailed examination of this consideration, however, would perhaps be better put off at least until additional experiments are described.

It should be emphasized that the above reasoning does not exclude heat content, evaporation cooling, or even a metabolic heater as influencing  $F$ . It merely concludes that neither heat content nor evaporation cooling nor in all probability a metabolic heater can *alone* account for the tonic response to  $T_a$  and  $H$ . The question was not whether these parameters are involved but whether any provides an adequate explanation.

*Relative Humidity, Difference in Vapor Pressure.* The second approach to the question of whether the dependence of  $F$  on both humidity ( $H$ ) and temperature ( $T$ ) can be reduced to a dependence on a single parameter is from the side of  $H$ . The proper  $T$ -dependent humidity parameter might eliminate any further dependence on  $T$ . For this purpose parameters involving vapor pressure ( $P_w$ ) would seem obvious choices. For inasmuch as the reasoning of the preceding section may be considered conclusive,  $H$  is affecting  $F$  to an extent which cannot be accounted for simply on the basis of heat content, evaporation cooling, or heat flow into or out of the sensillum. Under these conditions it is hard to see how there could be such an effect without water entering or leaving some part of the sensillum. That  $P_w$  as exerted only in the air streams does not eliminate the double dependence is clear from Figure 5. But  $P_w$  is not

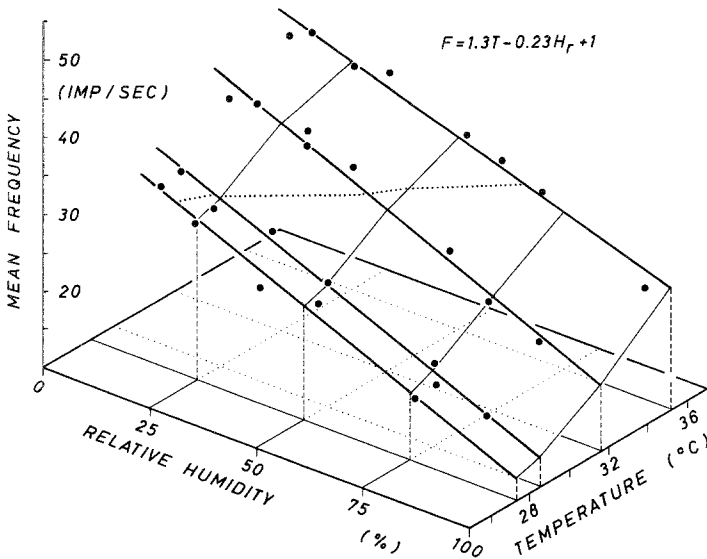


Fig. 7. Double linear regression and construction as in Figure 6, with same tonic responses here as a function of temperature and relative humidity. Position of individual points in Figures 5 to 7 all indicate that the frequency vs. moisture curves deviate from linearity. Their slopes tend to diminish with increasing dryness. Since in no case is the slope of either the F vs. H or of the F vs. T curves zero, each F is the response not to a single value of H or T or of both, but to a set of combinations of them. See equal frequency curve as example (heavy dotted diagonal)

exerted only in air. It also makes itself felt in aqueous solution and at the boundary between the solution and the air surrounding it. At this boundary  $P_w$  can approach its saturation value ( $P_s$ ), and the dependence of  $P_s$  on  $T$  far exceeds mere linearity. Assuming effective contact between water vapor in the air and the aqueous medium within the sensillum, then a  $P_w$ -gradient from the interior of the sensillum to the air outside could develop. By using  $P_s$  as an approximation for inside  $P_w$ , this situation would lend relevance to two parameters: the difference in vapor pressure ( $P_s - P_w$ ) and the ratio between the two ( $P_w/P_s$ ). The latter is by definition relative humidity ( $H_r$ ).

Figures 6 and 7 show that a clear  $T$ -dependence persists when  $F$  is plotted against either of these humidity parameters. Thus whether  $P_w$  were to turn out to affect  $F$  indirectly by altering the shape of the sensillum (hygrometer), or the ion concentration within it, or in some other way, there is a residual dependence on  $T$  beyond what  $H$  can account for alone. Regardless of how  $H$  has been expressed, the  $F$ -level is higher not only when the  $H$ -level is lower and  $T$  constant. At constant  $H$  the  $F$ -level is also higher when the  $T$ -level is higher. There does not appear to be any single parameter from the side of either  $H$  or  $T$  which eliminates this double dependence. Even if the  $T$ -dependent parameter,  $P_w$  internal to the sensillum (by approximation,  $P_s$ ), is substituted for  $T$  in all the preceding considerations, the response remains ambiguous. A given  $F$  corresponds not to a pair of values but to a set of

pairs, as indicated by the heavy dotted diagonal lines in Figures 5, 6, and 7. These relationships were confirmed in varying degrees by every receptor examined for tonic response.

Otherwise the difference in the plots is mainly in the slopes of the frequency-moisture curves for different temperatures. The tendency of these curves to converge appears to be least when moisture of the air is expressed as relative humidity. The significance of this finding seems limited however. For in contrast to the above relationships which are confirmed by each characteristic curve in which the dependence of frequency on either temperature or moisture alone was determined, these relationships can be examined only when both parameters involved are varied. As yet the number of receptors from which frequency-moisture curves for several temperatures were successfully recorded seems too small to permit the generalization that these curves tend to be most nearly parallel when Hr is taken as humidity parameter. Closer inspection also shows that these curves are only approximations anyway. Nevertheless, if these facts are borne in mind, the relationships shown in Figure 8 can be used to indicate the extent of ambiguity in the D-cell's response.

#### *Ambiguity-Bimodality*

The extent of ambiguity is indicated in Figure 8A, B. Here F is plotted as a function of T (in Fig. 8A) at various values of Hr between 0 and 100% and as a function of Hr (in Fig. 8B) at various values of T between 22.3 and 40 °C, both on the basis of the relationship,  $F = 1.3 T - 0.23 \text{ Hr} + 1$  from Figure 7. The T-axis has been extrapolated about 4° to either side of the domain measured. In this example, when  $T = 31.15^\circ\text{C}$  and  $\text{Hr} = 50\%$ ,  $F = 30 \text{ imp/s}$ . This value of F remains same when  $T = 22.3^\circ$  and  $\text{Hr} = 0$ , and when  $T = 40$  and  $\text{Hr} = 100\%$ . The example (Fig. 8A) shows a frequency range of  $\Delta F = 23 \text{ imp/s}$  encoding  $\Delta T = 17.7^\circ$  at constant Hr between 0 and 100%. Figure 8B shows the converse. The same  $\Delta F = 23 \text{ imp/s}$  encodes  $\Delta \text{Hr} = 100\%$  at constant T between 22.3 and 40 °C. It is not as though one section of the F spectrum were reserved for T and the other for Hr. The same spectrum encodes both. But  $\Delta F = 23 \text{ imp/s}$  represents a significant segment of the total F-range available for tonic responses (see below). Within this range T-variation can produce an error involving 100% Hr (total domain) and Hr variation can produce a 17.7° error in T, or roughly  $1/2$  the T domain not ultimately lethal to *Periplaneta*.

In another example, where the T domain is more extensive but from a smaller number of F vs. Hr curves than shown in Figure 7, the T-component is the same (0.134 imp/s/°C), but the Hr-slope is not as steep ( $-0.137 \text{ imp/s/\%}$ ). In relation to Hr-sensitivity, T-sensitivity is greater here. This situation is not greatly altered when the T and Hr slopes of all tonic recordings are averaged and their means compared ( $2.24 \pm 0.73 \text{ imp/s/}^\circ\text{C}$  and  $0.17 \pm 0.045 \text{ imp/s/\%}$ ). Unfortunately the averages also include recordings at only one value of T or  $P_w$  while the other was varied, and not every curve,  $F_{(\text{Hr})}$ , has a counterpart,  $F_{(\text{T})}$ . Nevertheless the examples all show either T or Hr as a considerable source of error with regard to the other, and if there is a lack of symmetry indicated,



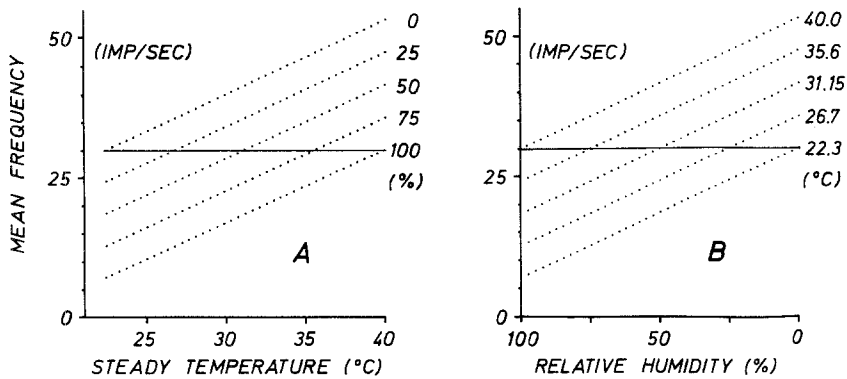


Fig. 8. Illustration of the extent of ambiguity of D unit tonic response as indicated by double linear regression approximating reaction to constant relative humidity and temperature. See Figure 7. In this case the same frequency spectrum ( $\Delta F=23$  imp/s) encodes all temperatures (T) between 22.3 and 40 °C (A above) and all values of relative humidity (Hr) from 0 to 100% (B above). Thus using tonic F as the indicator, T variations involving 17.7° can lead to an error of 100% in the Hr reading, and variation in Hr from 0 to 100% can lead to a 17.7° error in the T reading, a significant portion of *Periplaneta's* biological range. No matter which pair of parameters the D unit turns out to be responding to primarily (one from each of the two groups: Ha, Pw, Ps-Pw, or Pw/Ps; and Ps or T), the tonic response of a single D unit is in any case very ambiguous; at worst, bimodal

the dependence of F on T relative to its dependence on Hr is greater than that illustrated in Figure 8—a situation rather remarkable for a receptor identified as a D unit by reason of its phasic response to humidity.

Neither humidity nor temperature appears susceptible of elimination by reducing the two to a single parameter both outside the sensillum and of which both are a function. Because the error in F produced by either modality with regard to the other tends to be massive, the response is termed bimodal. There is however, an important limitation to the extent of ambiguity. F is highest where T is high and H is low, but with diminishing gain. There is little indication from any receptor encountered that continuous F over 65 imp/s could be sustained before lethal combinations of T and H are reached. Frequencies above this value would no longer represent responses to levels of T and H. They would be elicited only in combination with transients and signal other parameters such as rate of humidity change (Loftus, in prep.).

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