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CHANGES IN CHEMORECEPTOR SENSILLA ON THE MAXILLARY PALPS OF *LOCUSTA MIGRATORIA* IN RELATION TO FEEDING

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INTRODUCTION

The domes of each maxillary palp of fifth-instar larvae of *Locusta migratoria* (L.) bear about 370 chemoreceptor sensilla. Studies with the scanning electron microscope on these sensilla indicate that the distal pores are capable of being opened and closed (Blaney & Chapman (1969) on *Schistocerca gregaria* and, unpublished, on *Locusta*), and Stürckow, Holbert & Adams (1967) describe a similar phenomenon in chemoreceptor hairs of *Phormia regina*. The effect of closing would be to isolate the dendrites from the external environment and since these sensilla are important in food selection (Blaney & Chapman, 1970) closure might be expected to occur in relation to feeding. The possibility of such a relationship in *Locusta migratoria* was investigated by measurements of the electrical resistance across the tips of the palps and by electrophysiological studies on individual sensilla.

MATERIALS AND METHODS

Insects

All the experiments were carried out on male fifth-instar larvae of *Locusta*. These were taken from the normal laboratory stock at the Centre for Overseas Pest Research and used in the period 24 h to 3 days after ecdysis so that the results are not influenced by the immediate proximity of the moult. During the experiments larvae were kept at 28–31 °C.

Measurement of resistance

The measurement of electrical d.c. resistance across the cuticle was based on the technique of Scheie & Smyth (1968), using two electrolyte contacts on the external surface of the intact insect. Simultaneous measurements were made of the applied current, I, and the potential difference, V, developed between the electrodes in the two electrolyte contacts. A curve of V against I was displayed on the CRO screen as the current was adjusted in either direction by a potentiometer and from this the resistance was calculated using Ohm's Law. The changes of current direction in the course of each measurement ensured that polarization did not occur.

The electrodes were of silver/silver chloride in non-tapered 1 mm diameter glass

tubes filled with 0·1 N-NaCl solution. These were renewed daily to ensure that electrode potentials were less than 5 mV. The resistance of the electrodes varied from 2 to 9 KΩ.

Larvae were fed individually to repletion and then kept without food for 0, ½, 1, 1½, 2, 4, 6, 8 or 20 h before being anaesthetized lightly with carbon dioxide and waxed into position prior to testing.

Each larva was placed dorsal surface down in a plaster-of-Paris block and fixed with the legs outstretched by means of a beeswax–resin mixture. The maxillary palps were waxed to the mandibles and these were similarly fixed to the labrum. Thus the palp tips were immovably fixed, pointing upwards.

Measurements were made of the electrical resistance across the head and maxillary palps by putting an electrode over each palp so that the distal one-third of the terminal segment of each palp was immersed in electrolyte. After measuring the resistance across the intact palps, the electrodes were temporarily raised, the domes of the palps were cut off and the resistance was measured again. Subtraction of the second value from the first gave the electrical resistance for the two palp tips. The electrodes were washed out with fresh electrolyte between each measurement.

Measurements of resistance were also made across areas of sclerotized cuticle and of membrane which did not bear large numbers of sensilla. A drop of electrolyte was placed on the triangular membranous region on the inner distal angle of each front femur, or on either side of the mid-line of the mesosternum, and the electrodes were lowered to make contact with these drops. After the resistance had been measured the drop diameters were measured using an eyepiece micrometer. The electrodes were then pushed through into the haemocoel to measure the resistance of the electrodes and body fluids. Subtracting the second value of resistance from that of the first gives a value for the resistance of the two measured areas of cuticle in contact with the electrolytes.

Electrophysiological studies on individual sensilla

The larvae were restrained with wax in a manner similar to that described above and silver/silver chloride electrodes were used to detect, extracellularly, electrical changes within the sensilla. The indifferent electrode was inserted into the haemolymph of the head, near the base of an antenna, and connected via a back-off d.c. source and calibration source to earth. The recording electrode was inserted in a glass capillary which was tapered to a 10 μm tip and contained the stimulating solution of 0·1 M sodium chloride. Electrical signals were fed through a cathode-follower input valve and an a.c.-coupled preamplifier to be displayed on a Tektronix 502A CRO and recorded on a tape recorder (Thermionic T3000). For subsequent analysis the electrical phenomena were printed out using a U.V. recorder (S.E. 3006/DL) or photographed on oscillograph paper.

Chemoreceptor responses were obtained by placing the recording capillary gently over the tip of the sensillum or, if no response was obtained in this way, by gently pressing the capillary tip against the side of the sensillum tip before or after the sensillum had penetrated the stimulating solution. Mechanoreceptor responses were obtained by more vigorous manipulation of the sensillum tip, involving lateral movement of the whole sensillum.

A relative measure of conductivity through the tip of a sensillum was obtained by measuring the potential difference between the two electrodes while a calibration pulse was applied. The calibration pulse was a square wave, 50 mV peak to peak, with a frequency of 1 KHz. In most cases it was applied throughout the period of recording.

RESULTS

Resistance across cuticle

The total electrical resistance across the domes of the two maxillary palps decreased from about 120 KΩ immediately after feeding to 97 KΩ 2 h later (Fig. 1). Subsequently the resistance fluctuated between 85 and 100 KΩ until at least 20 h after feeding.

The electrical resistance across cuticle which was devoid of sensilla did not vary. Across the fully sclerotized cuticle of the sternum the resistance was constant at about 6 KΩ cm^{-2} irrespective of the state of feeding, and the resistance across the femoral membrane was similarly constant, although lower (Table 1).

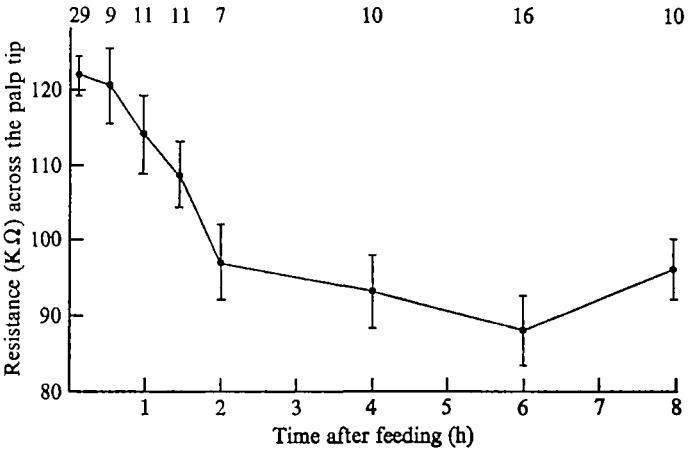

Fig. 1. Changes in resistance across the dome of the maxillary palp in relation to the time of feeding. Vertical lines indicate standard errors; numbers along the top show the numbers of insects on which measurements were made.

Table 1. *Resistance across the cuticle of the sternum and femoral membrane in relation to feeding*

	Sternum		Femoral membrane	
Hours after feeding	No. of measurements	Resistance (KΩ cm^{-2}), (mean ±S.E.)	No. of measurements	Resistance (KΩ cm^{-2}), (mean ±S.E.)
0	12	6·4±0·26	8	2·9±0·11
2	—	—	8	2·9±0·11
4	12	6·5±0·34	8	2·9±0·14
6	12	6·5±0·17	—	—

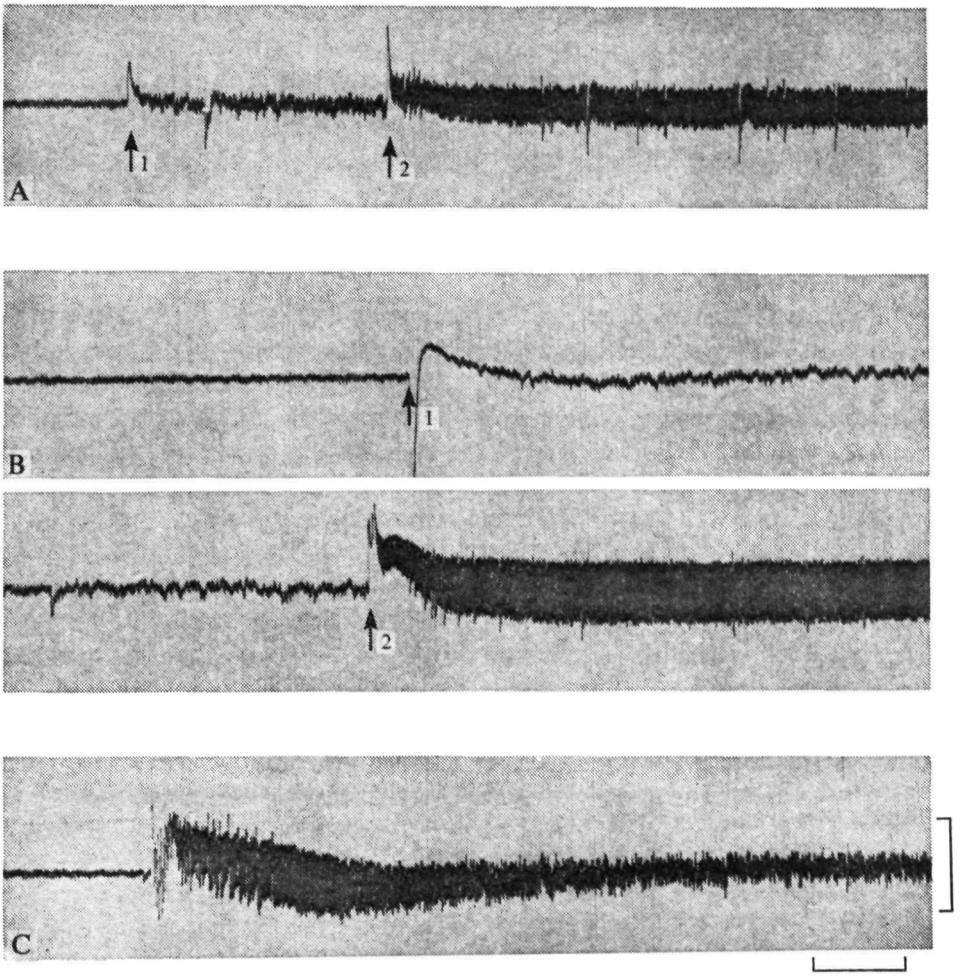

Fig. 2. A, B. Spontaneous changes in the amplitude of the calibration pulse passing through a sensillum. The electrode was placed over the sensillum at position (1) and a spontaneous change occurred in the record at position (2). In B the record is continuous from the upper to the lower trace. Notice the burst of action potentials following the increase in amplitude of the calibration pulse, but largely obscured by it. C. A progressive decline in the amplitude of the calibration pulse following a sharp increase produced by manipulation of the sensillum. Horizontal bar shows 0·1 sec, vertical bar 0·5 mV.

Studies on single sensilla

When the stimulating electrode was placed over a sensillum it commonly happened that no spikes were recorded and no calibration pulse would pass, but subsequently, in some cases, there was a sudden spontaneous increase in the height of the calibration pulse associated with a burst of spikes (Fig. 2A, B). Conversely, it occasionally happened that the height of the calibration pulse decreased with time (Fig. 2C).

Similar effects could be produced by very gentle manipulation of the sensillum with the tip of the capillary, the amplitude of the calibration pulse increasing from the noise level of 0·08 mV to a mean value of 1·04 mV (Fig. 3). Only 11 out of 279 sensilla

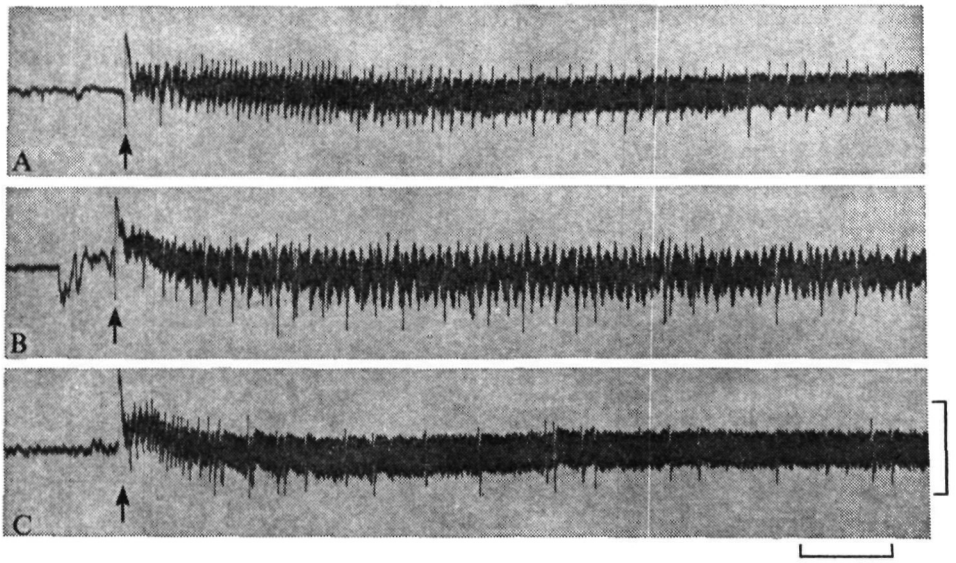

Fig. 3. A–C. Traces from individual sensilla which failed to respond to stimulation until they were manipulated by gentle movement of the stimulating electrode (↑). Notice the sharp increase in the amplitude of the calibration pulse and the associated burst of action potentials, largely obscured by the pulse. Horizontal bar shows 0·1 sec, vertical bar 0·5 mV.

Table 2. *Numbers of sensilla which failed to respond electrically when stimulated with a* 0·1 M-*NaCl solution, in relation to the period of food deprivation*

Period without food (h)	No. of insects tested	No. of sensilla tested	Sensilla failing to respond	
			No.	%
0	11	110	77	70
2	7	70	40	57
4	10	99	49	50

failed to respond and these may well have been different types of sensilla (Le Berre, Sinoir & Boulay, 1967).

This manipulation also produced a burst of firing from the neurones. This was not a mechanoreceptor response since distinct mechanoreceptor responses were only produced by more vigorous movement of the sensillum and were of greater amplitude (Fig. 4).

The frequency with which no response was obtained on first placing the electrode over a sensillum varied inversely with the period of food deprivation (Table 2), the difference between insects just after feeding and after 4 h without food being highly significant ($\chi_2 = 8{\cdot}31$, 1 d.f., $0{\cdot}01 > P > 0{\cdot}001$). There was, however, no indication of a higher conductivity across the tips of responding sensilla after longer periods of food deprivation (Table 3) or of any change in the spike frequency. Considering only sensilla with a calibration pulse amplitude of 0·8 mV or more, the average impulse frequency over the first second of stimulation was 45, 55 and 50 after 0, 2 and 4 h food deprivation respectively.

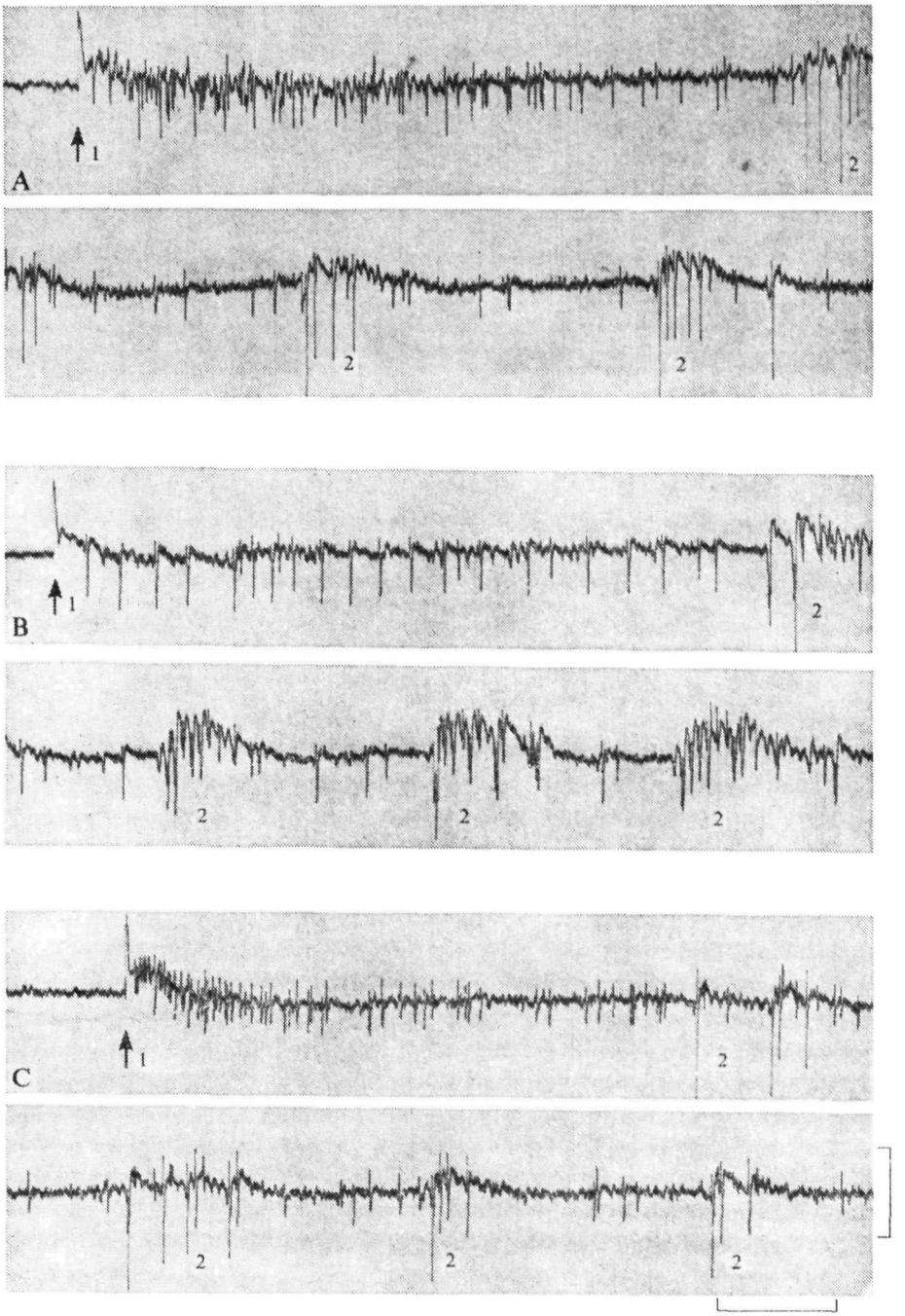

Fig. 4. A–C. Traces from individual sensilla showing, at position (1), the response to gentle manipulation by the stimulating electrode followed, at (2), by the response to more vigorous mechanical stimulation. In each case the records are continuous from the upper to the lower traces. Horizontal bar shows 0·1 sec, vertical bar 0·5 mV.

Table 3. *Calibration pulse amplitude across responding sensilla following different periods of food deprivation*

	Period without food (h)	No. of sensilla	% passing pulse of 0·4 mV or more	% passing pulse of 0·8 mV or more
Sensilla responding without manipulation	0	30	40	23
	2	29	24	3
	4	42	50	14
Sensilla responding after manipulation	0	78	92	92
	2	70	93	84
	4	81	91	88

DISCUSSION

The results indicate that changes occur in the resistance across the tips of the maxillary palps in relation to feeding. Comparable changes do not occur across areas of cuticle which bear few chemosensilla or none at all, suggesting that the changes in resistance of the palps reflect alterations in the battery of terminal sensilla. The studies on single sensilla indicate that such changes may occur since a sensillum will sometimes respond to chemical stimulation while at other times no response can be recorded. The frequency with which responses occur spontaneously, without manipulation of the sensilla, increases with the time since feeding, paralleling the changes in resistance and again suggesting that the sensilla are directly involved in these changes.

The mechanism by which this change in electrical resistance is brought about can only be inferred. Morita & Takeda (1957), working on tarsal receptors of *Vanessa* and Stürckow (1971) on chemoreceptors of *Calliphora*, suggests that a rise in resistance may be related to the presence of large amounts of viscous material which oozes from the tip of the sensillum. This material is also present in *Locusta*, but we have no evidence of any changes in it in relation to feeding. Further experiments (Bernays & Chapman, 1972) show that the increase in the measured electrical resistance is associated with changes in the haemolymph, and can be induced in insects deprived of food for 4 h by blood transfusions from newly fed insects. This seems to preclude the possibility of changes being caused by the accumulation of viscous material or food residues on the outside of the sensilla during feeding, and suggests that the effect is associated with some internal mechanism.

The rapidity with which changes in the amplitude of the calibration pulse occur, either spontaneously or after manipulation, suggests a sudden change in the sensillum which would be in keeping with the terminal pore opening or closing. The evidence from the scanning electron microscope (Blaney & Chapman, 1969; and unpublished) and from the photographs of Stürckow *et al.* (1967) shows that opening and closing can occur. A possible mechanism by which this might be brought about is discussed by Blaney, Chapman & Cook (1971).

The effect of closure of the sensilla would be to isolate the dendrites from the environment so that the input from these sensilla is minimized. Since each sensillum is associated with at least six neurones a total of at least 2220 (370 × 6) axons pass to the suboesophageal ganglion from the tip of each maxillary palp. Hence from the four

palps, maxillary and labial, a total of about 10000 axons is involved. The reduction in sensory input from these during the period immediately after feeding would prevent distraction at a time when further feeding is either not physically possible or is, perhaps, physiologically disadvantageous.

The fall in resistance in the 2 h after feeding is in concordance with the natural periodicity of feeding. Experiments with isolated fifth-instar larvae of *Locusta* have shown that isolated insects in a constant environment commonly feed at approximately 1 h intervals and only rarely fail to feed within 2 h of the last meal (Blaney, Chapman & Wilson, unpublished). The present data suggest that input from the terminal sensilla of the palps will be increasing rapidly during this period and it has been shown (Blaney & Chapman, 1970) that the maxillary palps have an essential role in normally feeding (i.e. non-starved) locusts.

In *Locusta*, however, active vibration of the palps virtually stops after feeding (Blaney & Chapman, 1970) so that stimulation of the palp sensilla is unlikely to occur in any case, and possibly the closure of the sensilla has some other function. It will result in some reduction in water loss, but it has been argued (Blaney & Chapman, 1969) that the conservation of water thus achieved by the insect as a whole is negligible. Perhaps conservation of the fluid bathing the dendrites is important. This fluid is produced by the neurilemma cell (Blaney *et al.* 1971) and wastage during food testing by the palps may be considerable. Hence a period of production and conservation after feeding may be necessary if the sensilla are to function adequately at the next feed.

The degree of opening, as measured by the amplitude of the calibration pulse, did not appear to vary in any regular manner with the period of food deprivation (Table 3), nor were changes observed in the average spike frequency of open sensilla. This contrasts with the situation in *Phormia* where Omand (1971) observed changes in the input from individual sensilla in relation to feeding. Possibly a similar overall effect is produced in *Locusta* by changes in the number of sensilla responding, rather than by changes in individual sensilla.

A number of components contribute to the measured resistance across the tip of a palp. These are: the sclerotized shaft of the terminal segment of the palp, the membranous dome at the tip of the palp, the sensilla, and the openings of the sensilla. The relative areas of the first three components are 45:35:30 and an estimate of the resistance with all the sensilla closed, assuming the resistance across the closed tips to approach infinity, can be based on a mean of the measured values/cm^2 for sclerotized and membranous cuticle. Deviations in the measured value of palp-tip resistance from this estimate will result from the opening of sensilla. By treating the open sensilla as resistances in parallel with the cuticle, a mean estimate of the resistance across the tip of a single open sensillum can be calculated.

The estimated resistance of a single open sensillum based on the palp resistance immediately after feeding, with 30% of the sensilla open, was 19 MΩ, while after 4 h without food, with 50% of the sensilla open, the value was 21 MΩ. These are in the same range as the values obtained by other workers on chemosensilla. Morita & Takeda (1957) recorded values of 20–100 MΩ from the tarsal receptors of *Vanessa*, while Wolbarsht (1958) and Stürckow (1971) obtained values of 20–80 and 27–62 MΩ from the labellar hairs of *Phormia* and *Calliphora* respectively.

SUMMARY

1. The electrical resistance across the tips of the maxillary palps of *Locusta migratoria* is high immediately after a feed, but falls to a steady level in about 2 h after feeding.

2. Only a small proportion of the individual sensilla on the domes of the palps respond to chemical stimulation immediately after feeding, but this proportion increases with the period of food deprivation.

3. Gentle manipulation of the tips of non-responsive sensilla causes them to respond.

4. It is suggested that the terminal pores of the sensilla can open and close and that the resistance across the tips of the palps varies inversely with the number of open sensilla.

5. The possible significance of the changes is discussed.

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