

## RECEPTOR POTENTIALS RECORDED FROM SENSILLA BASICONICA ON THE ANTENNA OF THE SILKWORM LARVAE, *BOMBYX MORI*

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Several authors have reported on electrical activities of antennae of insects in response to various olfactory stimuli (Boistel, 1953; Roys, 1954; Boistel, Lecomte & Corabœuf, 1956; Schneider & Hecker, 1956; Schneider, 1957). Dethier (1941) studied the sensilla of the antenna of lepidoptera larvae, and assumed that the 'large' sensilla basiconica were olfactory organs.

One of the authors (Morita, 1959) recorded slow potentials directly related to occurrence of impulses (i.e. receptor potentials) from the side wall of the contact chemosensory hair on the labellum of the blow-fly, *Calliphora vomitoria*. The 'large' sensilla basiconica on the antennae of lepidoptera larvae are far smaller than the labellar chemosensory hair, but not so small as to prevent application of the method available for the labellar hair. In the present study, using this method, electrical activities of the sensillum basiconicum of the silkworm larva were qualitatively analysed.

### METHODS

As described by Dethier (1941), the antenna of lepidoptera larvae consists of three segments: the first segment from the base of the antenna contains four sensilla campaniformia; the second has one sensillum campaniformium, two sensilla trichodea and three sensilla basiconica (two large and one small); the third has one sensillum styloconicum and three sensilla basiconica (one large and two small). Dethier (1941) described the large sensillum basiconicum as innervated by more than twenty sensory neurons. This type of sensillum on the antenna of the silkworm larva was used in the present study. It may be assumed that the histological structure of this sensillum of the silkworm larva is the same as reported by Dethier, because this larva belongs to the order Lepidoptera.

The arrangement for recording electrical activities of chemosensory neurons was almost the same as used by Morita (1959). The head of the fifth-instar larva of the silkworm, *Bombyx mori*, was isolated at its base. As shown in Fig. 1, a platinum wire, which was thrust into the base of the antenna from the cut end, was grounded through a balancing voltage (*BV*) and calibration pulse sources (*CP*). A glass capillary (*Cr*) filled with 0.65M-NaCl with a tip diameter of about  $1\mu$  was used as a recording active electrode, the tip of which was inserted into a 'large' sensillum basiconicum on the antenna by means of a micromanipulator. Electrical activities of the sensillum

were fed into an amplifier through a platinum wire which was dipped into the 0.65 M-NaCl solution in the capillary (*Cr*).

By means of a hydrostatic pressure, air which flowed through an activated carbon filter and a flexible vinyl tube was directed from an opening of a glass tube (*Ca*, about  $50\mu$  in diameter) toward the tip of the sensillum. Although the vinyl tube was washed with running water for a long time after cleaned with detergent, it might not have been completely odourless. For this reason the response to a given chemical stimulus was always confirmed by a control experiment as described below. The pressure, which drove the air out, was produced by water at different levels in two communicating bottles. The rate of air flow could be regulated with a pinch cock which was fitted to the vinyl tube. The tip of the 'air-tube' (*Ca*) was placed at about 0.3 mm. from the sensillum. A stimulating capillary (*Cs*),  $10\text{--}30\mu$  in tip diameter,

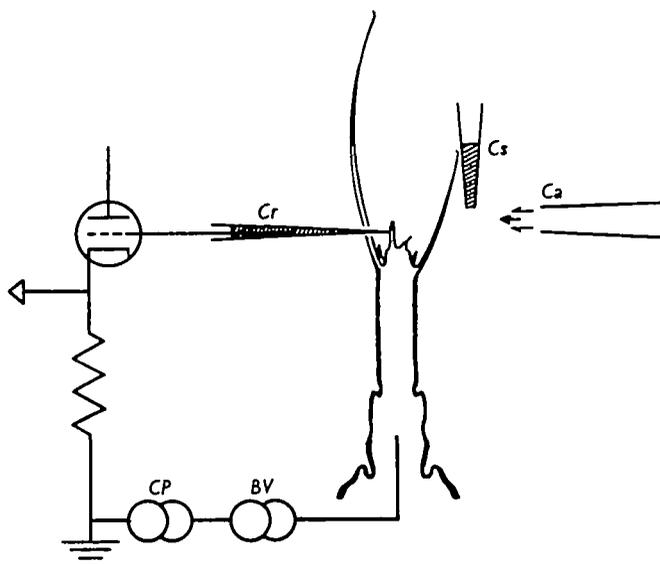


Fig. 1. Experimental arrangements. *Cs*, stimulating capillary containing a stimulus substance, mounted on the moving iron of an electromagnet; *Ca*, capillary used to blow air to the sensillum; *Cr*, recording capillary; *CP*, calibration pulse source; *BV*, balancing voltage source.

was placed near the tip of the 'air-tube' (*Ca*) perpendicularly to the air current as it flowed on to the sensillum. The olfactory stimulation was applied by exposing the tip of capillary (*Cs*) filled with an odorous substance to the air current with the aid of an electromagnet, while an empty capillary was used in control experiments. The beginning and the end of an electric current through the electromagnet are indicated by arrows in each record. The chemical stimuli therefore became operative after some delay relative to these marks, which depended upon the rate of air flow.

Odorous substances applied were *n*-hexanol,  $\beta\gamma$ -hexenol, *n*-butylaldehyde, acetic acid, butyric acid, etc. The essential oil of mulberry leaves contains all of these substances except *n*-hexanol (Watanabe, 1958). It is known that  $\beta\gamma$ -hexenol is strongly attractive to the silkworm larvae (Watanabe, 1958). All stimulating substances were used without being diluted with any solvent. The strength of the stimulus was

therefore not strictly controlled, but this fact was not serious for the present qualitative study of the chemoreceptors.

The head of the d.c. amplifier used was composed of a 12AU7 cathode follower, its grid current being less than  $10^{-12}$  A. The electrical responses of the sense cells, appearing on repeated sweeps of an oscilloscope, were photographed on a running film. In each record a calibration pulse of +1 mV. (i.e. positive with reference to earth) was added just before the chemical stimulation. In most cases the rectangular wave of 60 cyc./sec. was also recorded simultaneously, and served for the control base-line as well as the time base.

All experiments were performed at room temperature, 26–29° C., and at relative humidity 80–85 %.

## RESULTS

### *Spike potentials*

When the tip of the recording electrode was inserted into the sensillum, a sudden drop of potential of a few hundred millivolts appeared and spike potentials were observed. In Fig. 2 some of these spikes are shown. These spontaneous spikes were

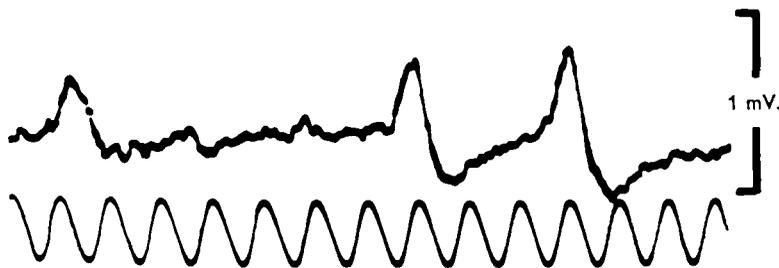


Fig. 2. Spikes recorded with an electrode thrust into a sensillum basiconicum.  
Time base, 1000 c/s.

not different in shape from those occurring upon chemical stimulation. It should be noticed that they are diphasic, positive to negative. This polarity is the same as observed in contact chemosensory hairs on *Calliphora labella* (Morita & Yamashita, 1959*b*; Morita, 1959).

### *Slow monophasic potential*

When the tip of a sensillum was exposed to a current of pure air for a brief period an increase in impulse frequency accompanied by a slow negative potential was recorded. In Fig. 3A the beginning of this response is shown on the first sweep, and after cessation of the air current the slow potential is seen to return to the initial level (at the beginning of the fourth sweep). As the sensillum was again exposed to a continuous air current, the base-line and the frequency of impulses attained a new steady level. This steady state was not affected at all by exposing the tip of a dry capillary to the air current (Fig. 3B). On the other hand, a slow negative potential and an increase in impulse frequency were induced if a capillary whose tip was filled with *n*-hexanol was used (Fig. 3C). The impulse frequency and the slow potential returned to their initial level when the capillary was withdrawn from the air current. From these results it may be assumed that the slow potential and the increase in

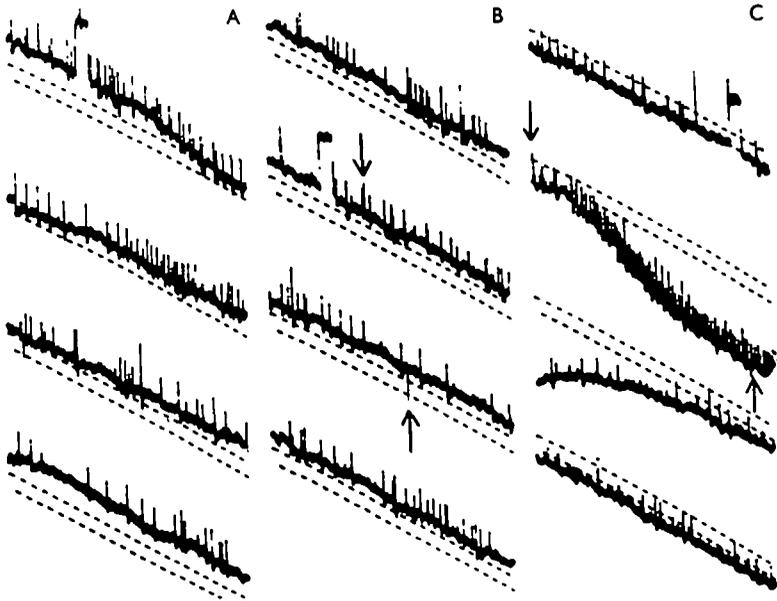


Fig. 3. A, air blown over the sensillum (from the middle of first sweep to the end of third sweep). A clean dry capillary (B, as control) and a stimulating capillary containing *n*-hexanol (C) were exposed to the air current. Arrows show 'on' and 'off' of an electric current through the electromagnet on which the stimulating capillary was mounted. Rectangular pulses of 60 cyc./sec. served also as control base-line. Calibration of rectangular pulse in each record, +1 mV. Unless stated otherwise subsequent figures are presented in the same way.

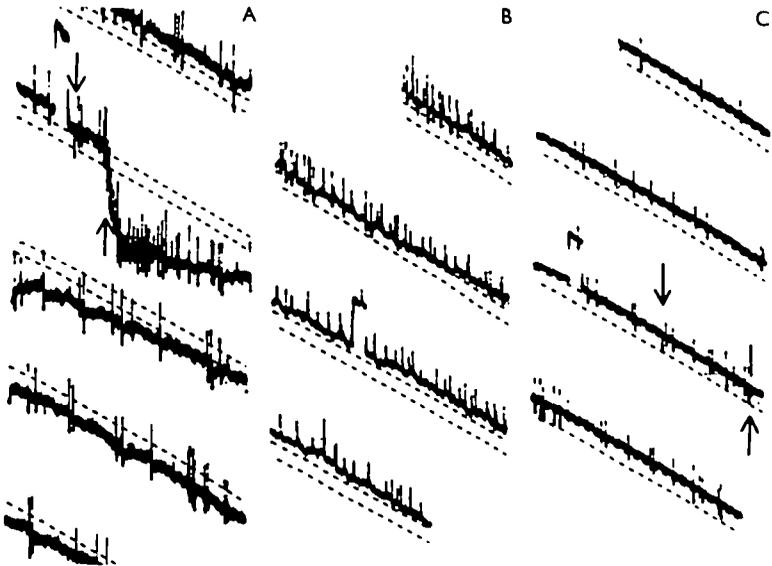


Fig. 4. Application of sulphuric acid to the tip of the sensillum. A, responses to *n*-hexanol before application. B, deformation in shapes of spikes immediately after application. C, responses to *n*-hexanol after application.

Impulse frequency were responses to chemical stimulation. The results shown in Fig. 4 also support this assumption. After the response to *n*-hexanol was ascertained to exist (Fig. 4A), the tip of the sensillum was touched with sulphuric acid, and then impulses became monophasic and showed a temporary increase in frequency (Fig. 4B). Thereafter, no response to *n*-hexanol was seen (Fig. 4C) except small spikes which were not related to chemical stimulation, and the origin of which we could not trace. Even then, another sensillum on the same antenna responded normally to *n*-hexanol.

There was no significant difference between electrical responses to *n*-hexanol (Fig. 5B) and to  $\beta\gamma$ -hexenol (Fig. 5C). Strictly speaking, however, it was almost impossible to compare their effectiveness because of the difficulty in accurate regulation of the stimulus strength.

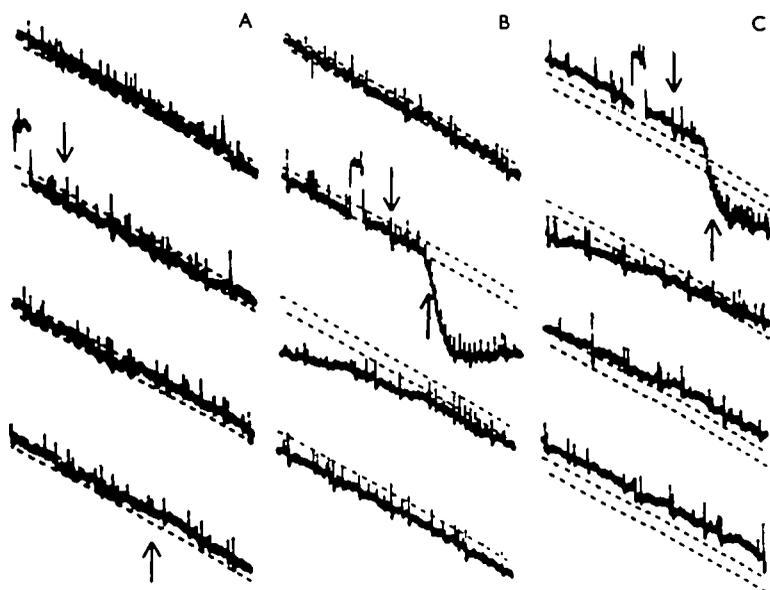


Fig. 5. Responses of a sensillum to  $\beta\gamma$ -hexenol and to *n*-hexanol. A, dry capillary (control); B, *n*-hexanol; C,  $\beta\gamma$ -hexenol.

Thus the negative slow potential was accompanied by an increase in impulse frequency in the antennal sensillum of the silkworm larva as observed in the labellar chemosensory hair of the fly (Morita, 1959). At the same time it should be noticed that impulses in the sensillum were discharged without chemical stimulation at a much higher frequency than in the labellar hair.

#### *Slow diphasic potential*

In many cases *n*-butylaldehyde induced responses of the same type as described above. In some cases, however, especially with strong stimulation, it provoked a different type of response. Fig. 6 (continuous from *a* to *c*) shows an example of this type which was recorded from the same sensillum as used in Fig. 3. In order to show the individual impulses evoked by the stimulus, a part of the record in Fig. 6 was

enlarged and is presented in Fig. 7. The potential declined slowly during the chemical stimulation, but after cessation of the stimulation it ascended gradually to a more positive level than the initial level. While the negativity was maintained, spikes (which were smaller than those evoked by *n*-hexanol) increased in frequency. Except for this period there was no detectable difference in impulse frequency. As can be seen in Fig. 7, small spikes decreased in height with an increase in sustained negativity, and seemed to be masked by noise under deep negativity, gradually growing up again to the original height with the recovery from the negativity. This observation may be explained by the relation established between the strength of the applied electric current and the spike height in contact chemosensory hairs of some insects (Morita & Takeda, 1959; Morita, 1959).

Slow diphasic potentials were recorded on stimulation by some other substances such as butyric acid, acetic acid, etc. An example of responses to acetic acid is shown

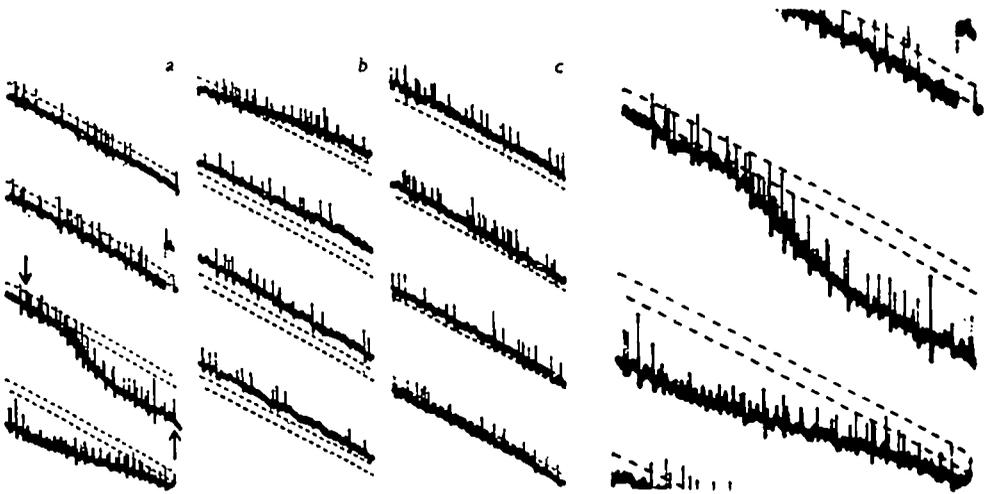


Fig. 6

Fig. 7

Fig. 6. Responses to *n*-butylaldehyde of the same preparation used in Fig. 3. Record continues from *a* to *c*.

Fig. 7. Enlarged photograph of a part of the record in Fig. 6, showing the individual impulses evoked by the stimulus.

in Fig. 8: *a*, *b* and *c* in A are the records obtained at the periods *a*, *b* and *c* in B, respectively, which represents the whole course of the slow potential. It was impossible to indicate the time of break of the electric current through the electromagnet because of the disappearance of the artifact. However, it must have been near the middle of the third sweep in A*a*, as judged from reading of the dial in an apparatus for current supply. As shown in this figure, the sustained positivity was much larger in amplitude and much longer in duration than the negativity maintained during stimulation. It was also noticeable that impulse frequency depended upon the rate of change in slow potential rather than upon the magnitude of the potential; that is, impulses increased or decreased in frequency with a fall or a rise of the slow potential, respectively. This tendency was generally observed in the responses to other stimulating substances. An attempt was made to study the nature of this after-positivity later in the same

year, but then all preparations responded without the after-positivity except one responding to acetic acid.

In responses to *n*-hexanol and  $\beta\gamma$ -hexenol an increase in impulse frequency was always accompanied by an increase in slow negativity, without exception. This result may be explained by the assumption that the receptor potential evoked near the tip of a sensillum initiates impulses somewhere near its base (cf. Morita, 1959). Diphasic slow potentials cannot be interpreted as simply as above. Moreover, it was observed that impulses increased in frequency during the slow monophasic positive potential. Fig. 9 shows an example of this sort, where *n*-butylaldehyde was applied. Record A

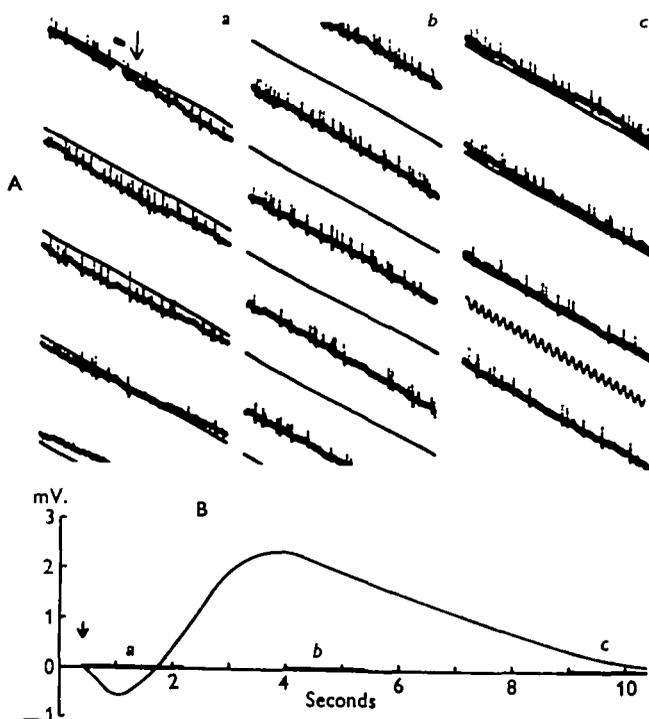


Fig. 8. Responses to acetic acid. Record A shows three parts of the whole response which is plotted in B. Thick lines *a*, *b* and *c* in B correspond to *a*, *b* and *c* in A, respectively. Calibration pulse, +1 mV. Time base, 60 cyc./sec. An electronic switch was used for obtaining dual beams, and one of the beams served as a control base-line.

in this figure was obtained with a stimulating capillary of about  $10\mu$  in tip diameter. The slow negative deflexion was maintained while impulses increased in frequency, and no after-positivity was seen. Thereafter, when the stimulating capillary was replaced by another whose tip diameter was  $25\mu$ , the slow positive deflexion appeared, being accompanied by an increase in impulse frequency (Fig. 9 B). Since these results were taken from the same sensillum, it may be assumed that the difference in response between records A and B was due to a difference in stimulus strength. At the same time, these results suggest that *n*-butylaldehyde might be excitatory for one group of chemosensory neurons and inhibitory for another group in the same sensillum. Further discussion will be given below.

*Effect of anaesthetics*

It was observed by Schneider (1957) that impulses recorded from the antennae of the silkworm moth were abolished by ether or chloroform vapour. In the present study impulses were never blocked with a capillary of less than  $100\mu$  in tip diameter containing ether or chloroform, no matter how long the exposure. Ether or chloroform, having a low boiling point, formed a droplet at the capillary tip, and this droplet disappeared because of rapid evaporation; then, another droplet was formed and disappeared in turn and this process was repeated. Fig. 10 (continuous from *a* to *c*) is a part of a record obtained during such an experiment with chloroform. In this figure the arrows indicate the moments when a droplet began to be formed and when the

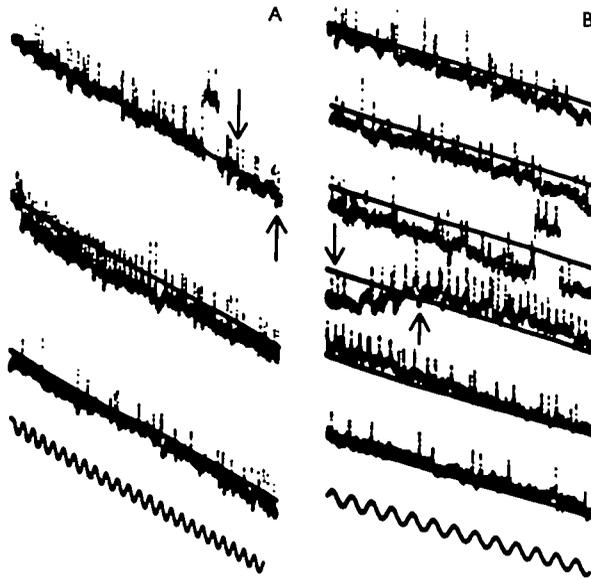


Fig. 9. Responses of the sensillum to *n*-butylaldehyde. In record A the tip diameter of the stimulating capillary was about  $10\mu$ ; in record B, about  $25\mu$ . Time base, 60 cyc./sec. The recording equipment as used in Fig. 8.

droplet disappeared. The sustained negativity coincided exactly with the formation of the droplet, being accompanied by trains of impulses. A more important fact in this record was alternation in occurrence of trains of impulses; after disappearance of the droplet the sustained negativity returned to the initial level, and as it did so a single train of impulses began to appear. A high frequency of impulses immediately after the disappearance of the droplet and its subsequent decline indicated that this single train of impulses was initiated by the disappearance of the droplet. From this result it may be assumed that chloroform vapour depolarized the receptor membranes of one group of chemoreceptors and initiated impulses in them. On the other hand, the same vapour may have hyperpolarized the receptor membranes of another group; after disappearance of the droplet an after-negativity might initiate impulses in this group of receptors, as observed in labellar chemoreceptors of the fly (Morita & Yamashita, 1959*a*). In fact, concentrated vapour of chloroform induced a positive,

potential which was maintained during the exposure, while the discharge of impulses was depressed. Fig. 11 (slow potential only was d.c. amplified, and spikes were a.c. amplified) shows a response of a sensillum to a strong stimulation by chloroform vapour. In this case a cotton wick, soaked in chloroform, was brought extremely close

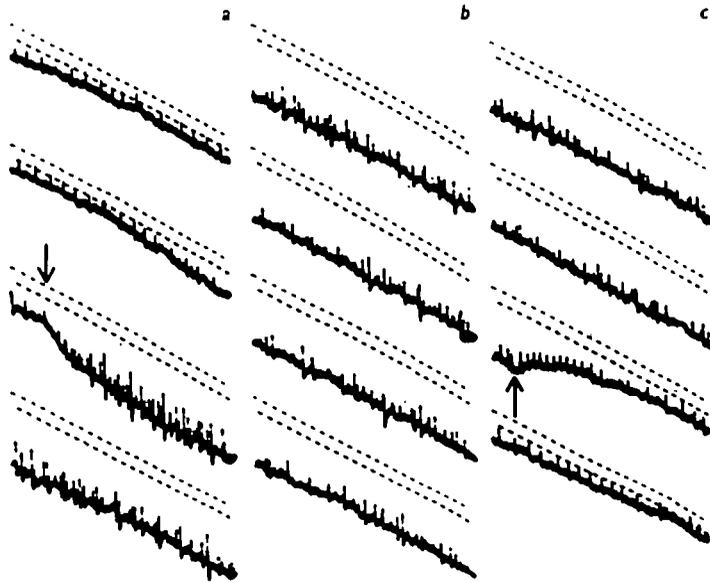


Fig. 10. Response evoked by exposing the capillary containing chloroform to the air current blowing to the sensillum (continuous from *a* to *c*). Arrows, ↓ and ↑, indicate the start of the formation and the disappearance of a chloroform droplet, respectively.

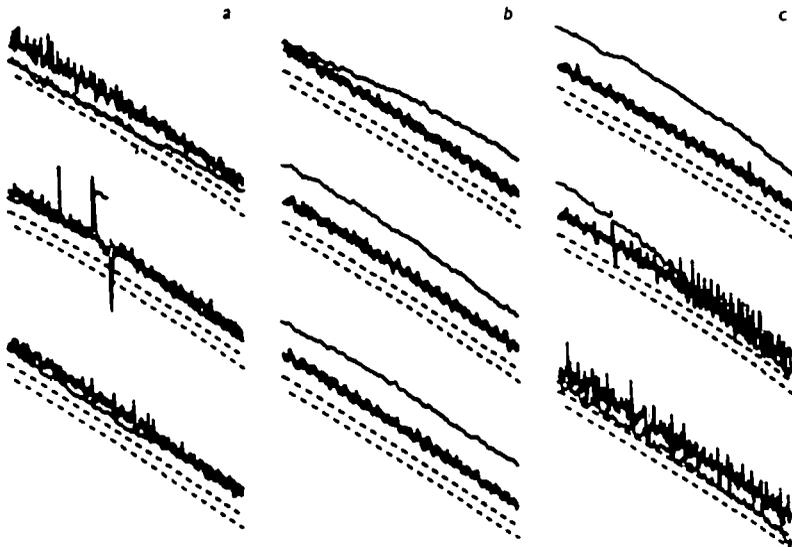


Fig. 11. Potential changes in the sensillum when a cotton wick soaked in chloroform was brought close to its tip for a short time. Continuous from *a* to *c*. Slow potential and impulses were separately recorded. Calibration pulses of +1 mV. are shown on second sweep in *a*.

to the sensillum for a short time. During the sustained positivity there was no train of impulses, suggesting that this positivity represented hyperpolarization of the receptor membrane.

#### DISCUSSION

Schneider (1957) recorded slow potential changes from antennae of the silkworm moth induced by olfactory stimulation, with a capillary electrode inserted into the antenna; he called this slow potential 'electro-antennogram', and considered it to be made of a number of components. Discussing the relation between the electro-antennogram and the impulse, he pointed out that the peak of impulse frequency did not coincide with that of the electro-antennogram. It was also assumed in the present study that the recorded slow potential was a complex of a few groups of receptor potentials. However, it may be possible to say from the present results that the negative receptor potential augmented the rate of impulse discharge.

As already described, the structural situation seems to be the same both in labellar chemosensory hairs of the fly and in sensilla basiconica of the silkworm larva. In the present paper it has been shown that electrical responses are also similar in the two kinds of sensilla if the same recording arrangement is used. That is to say: (1) Spikes were diphasic, positive to negative. (2) A chemical stimulus generally induced a slow negative potential accompanied by an increase in impulse frequency. (3) A slow positive potential, which could be evoked by application of anaesthetics, abolished all impulses. These results may be explained, as described for the labellar chemoreceptors of the fly (Morita, 1959; Morita & Yamashita, 1959*a*), as follows: chemical stimulants depolarized or hyperpolarized the receptor membranes which were located near the tip of the sensillum. These changes in the membrane potential controlled the rate of discharge of impulses which were initiated somewhere near the base of the sensillum. The initiated impulses were conducted toward the tip of the sensillum as well as to the nervous centre. Accordingly, they were recorded as diphasic (positive to negative) impulses with an active electrode inserted into the sensillum. On application of sulphuric acid to the tip of a sensillum chemosensory impulses became monophasic before the sensillum became completely unresponsive to chemical stimulation (Fig. 4). This is reasonable, because impulse conduction must be blocked at the point near the tip sooner than at the basal region (cf. Morita, 1959).

On the other hand, some of the present records showed characteristics which were not observed in the labellar hair: (1) Impulses sometimes increased in frequency while the slow monophasic positive potential was induced. (2) In some cases a chemical stimulus gave rise to a slow diphasic potential; even at a phase near the peak of the positivity impulse frequencies were as high as at the peak of the negativity. These characteristics might result from the existence of a large number of chemosensory units in the single sensillum on the antenna, and could be explained assuming a complicated combination of a hyperpolarization followed by an after-negativity and a depolarization followed by an after-positivity. The after-positivity was not detected in the labellar chemoreceptor, and the high level of spontaneous activities in the sensilla basiconica might be responsible for it.

## SUMMARY

1. The electrical responses of the sensilla basiconica on the antennae of the silkworm larva, *Bombyx mori*, have been studied by the microcapillary electrode technique.
2. Olfactory stimulation generally evokes a slow negative potential accompanied by increase in impulse frequency; but some agents evoke a slow diphasic potential, from negative to positive.
3. Strong vapours of anaesthetics evoke a slow positive potential with decrease in impulse frequency.
4. By comparison with the case of the labellar chemosensory hair of the blowfly, these results on the silkworm are explained in terms of depolarization or hyperpolarization of the receptor membrane.

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