ELECTRICAL ACTIVITY IN THE CHEMORECEPTORS OF THE BLOWFLY

I. Responses to Chemical and Mechanical Stimulation*

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ABSTRACT

The electrical responses of the neurons associated with the various types of chemosensory hairs of the blowfly, *Phormia regina* Meigen, following stimulation by chemical and mechanical means have been studied. The singly innervated chemosensory hairs on the ovipositor, maxillary palpi, and antennae respond vigorously to chemical stimulation, but not to mechanical stimulation. The triply innervated chemosensory hairs on the labellum, tarsus, and wing have two neurons which respond only to chemical stimuli. The third neuron responds only to mechanical stimulation. The differential responses of the two chemosensory neurons to various chemical stimuli following the removal of the tip of the hair suggest that the structures responsible for chemoreception are located throughout the distal processes of these neurons. The response of the third neuron to mechanical stimulation is similar to the response recorded from the neuron associated with one type of tactile hair which responds to motion and not to steady deformation. Recordings have been made from the neurons associated with purely tactile hairs using the cut hair as an extension of the micropipette. The mechanosensory neuron of the wing chemosensory hair is capable of responding at the rate of at least 600 impulses per sec. and may serve to indicate changes in air flow over the wing surfaces during flight to enable the fly to correct the wing camber and attack angle.

INTRODUCTION

The blowfly, *Phormia regina*, Meigen, is known to respond behaviorally to mechanical stimulation of its labellar and tarsal chemoreceptor hairs (Dethier, 1955). Recent electrophysiological investigations by Hodgson and Roeder (1956) have suggested that the neurons responsible for chemoreception in the labellum also respond to mechanical stimulation. This is apparently a violation of the law of the adequate stimulus (Ruch, 1946). This law may be para-

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phrased as follows: Sense organs are quite sensitive to one particular form of adequate stimulation and relatively insensitive to all other forms. This response of a neuron to both mechanical and chemical stimulation in the way described by Hodgson and Roeder (1956) is, therefore, quite unusual and unexpected.

The present study was undertaken to investigate more thoroughly which neurons in the chemoreceptor hairs were sensitive to mechanical stimulation. During the course of the study, we investigated all the types of chemoreceptors known on the blowfly by either behavioral or structural criteria that our methods permitted. These include the receptors on the labellum, tarsi, wings, maxillary palpi, ovipositor, and antennae. We have been able to show that the chemosensory hairs on the first three organs contain neurons that are stimulated specifically by mechanical deformation and that these neurons are similar to one of the types of neurons found in purely tactile hairs. We have also been able to show that the chemosensory neurons are quite insensitive to mechanical stimulation.

The chemoreceptor hairs can be put into two distinct morphological groups, those innervated by one neuron, and those innervated by three or more. The antennae, ovipositor, and maxillary palpi contain singly innervated hairs; while the labellum, tarsi, and wings have multineuronal hairs.

On the antennae the singly innervated chemosensory hairs may arise directly from the surface, or may have their bases in olfactory pits. The olfactory pits are located on the proximal end of the ventral surface, while the surface hairs occur mainly on the distal ventral surface of the antennae (Liebermann, 1926). Olfactory functions have been ascribed to the antennae on the basis of behavioral experiments by many workers (Dethier, 1954); however, no distinct function has been ascribed to either type of hair. Frings and Frings (1949) were not able to elicit any type of behavioral response by bathing the antennae with sugar or salt solutions. It is not certain, however, that they were making contact with the chemosensory hairs.

The palpi have hairs similar to the surface type of hairs on the antennae. Lowne (1890–95) classed them as chemoreceptors as did Wesché (1905). Frings and Frings (1949) were unable to demonstrate any behavioral response to contact chemical stimulation although Dethier (1952) has shown that the palpi have an olfactory function similar to that of the antennae.

Arab has suggested the existence on the ovipositor of hairs resembling those on the antennae and palpi. They are located among the longer, thicker hairs on the anal scales. Barton Browne has demonstrated that the ovipositor responds behaviorally to an olfactory stimulus administered to it during oviposition, but he has not identified the receptor responsible for the reaction.

Each labellar chemosensory hair has at its base a sac of five cells. The sac is surrounded by a basement membrane continuous with both the basement

membrane of the lemnoblast or neurilemma and the basement membrane of the hypodermis. A pigment cell layer with many lacunae usually surrounds the basement membrane of the sac. Two of the sac cells, the trichogen or hair-forming cell and the tormogen or socket-forming cell, are non-nervous. The other three cells in the sac are bipolar neurons which are approximately equal to each other in size. Each of these cells has a distal process which can be traced to the socket of the hair. There, one of the fibers seems to terminate. The other two enter the lumen of the hair and continue to a small papilla at the extreme tip of the hair (Dethier, 1955). The hair, except in the region of the papilla, is covered with a hydrophobic wax the electrical resistance of which is as high as that of glass.

Behavioral studies have shown that both chemical and tactile stimulation of a single labellar hair will elicit a response from the fly. Since either modality can be adapted out without affecting the other, there is a strong presumption that separate neurons are responding to each type of stimulation (Dethier, 1955).

Dethier (1955) postulated that two of the neurons—those whose distal processes terminate at the tip of the hair—are concerned with chemoreception. These two chemosensory neurons would respond differentially to various chemicals to indicate their acceptability or unacceptability. The third neuron, which terminates in the area of the socket, would respond to tactile stimulation.

In structure the tarsal chemoreceptor hairs are quite similar to the labellar hairs except that no pigment cell layer is present (Grabowsky and Dethier, 1954). Both chemical and tactile stimulation evoke behavioral responses. The behavioral response to chemical stimulation is analogous to that caused by chemical stimulation of the labellar hairs. The response to tactile stimulation differs; mechanical stimulation of a tarsal hair causes relocation of the leg; similar stimulation of a labellar hair causes a positive feeding response (Dethier, 1955).

Although the sense organs in the wings of Diptera have been described by many histologists during the last 100 years (Hicks, 1857), one type seems to have escaped detection. The costal vein has on both ventral and dorsal surfaces a row of hairs which project perpendicular to the plane of the wing proper. These hairs are set back and separate from the short thick spines which line the leading edge of the wing. Externally these hairs are similar in size and appearance to the type A tarsal chemoreceptors (Grabowski and Dethier, 1954). There are approximately forty of these hairs on each surface. They are quite evenly spaced from the proximal position of the vein to its junction with the subcosta. From this point to the distal end of the marginal vein the hairs are sparser but still evenly spaced. Fig. 1 shows a photograph of the ventral surface of the wing in the region where the subcosta

joins the costal vein. The ventral hairs are located alternately with the dorsal hairs. Male and female flies seem to have the same distribution of hairs. Each of these hairs at its base apparently has a cluster of cells, at least two of which send processes up into the hair.

Zácwilichowski (1933) has described similarly innervated hairs on the wings of the scorpion fly, *Panorpa communis*. However, these hairs are not found in all types of insects; for example, the three species of Diptera that Zácwilichowski (1930) examined, *Silvius vituli*, *Hoplodonta viridula*, and *Drosophila fenestralis*, apparently do not have this type of wing hair. Our own

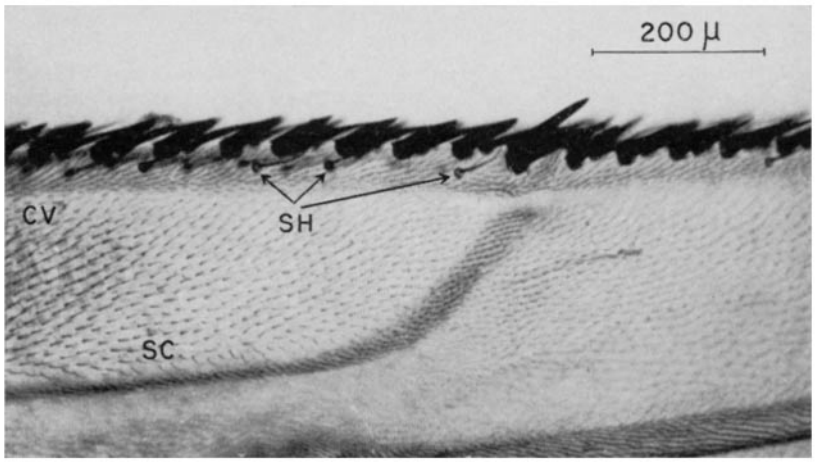

FIG. 1. Ventral surface of *Phormia* wing. *SH*, sensory hairs; *CV*, costal vein, *SC*, subcosta.

examinations show that *Drosophila melanogaster* and *Sarcophaga sp*? do have these hairs, and a more careful examination may disclose a wider distribution than is now known.

Since these wing hairs are so similar in structure to those on other parts of the body already known by behavioral and electrophysiological examination to be chemoreceptors, it is not unlikely that these hairs also are chemoreceptors.

Singly innervated tactile hairs are located over most of the fly's body. We have examined many of them in order to correlate their responses with those of their more complex neighbors.

Methods

All the experiments described here were performed on laboratory-cultured *Phormia regina* Meigen. Much of the preliminary investigation of the receptors on the antennae, maxillary palpi, wings, and ovipositor, was carried out on the flesh-fly *Sarcophaga sp*?; however, only minor differences between the two species were seen.

The usual recording technique has been described briefly elsewhere (Wolbarsht, 1957). It is a modification of a method first described by Hodgson, Lettvin, and Roeder (1955). A 2 to 50 μ glass capillary filled with some conducting solution was brought into contact with the exposed end of the chemoreceptor or tactile hair. A silver chloride–coated silver wire in contact with the conducting solution and one input lead of the preamplifier allowed the capillary to be used as the recording electrode. The indifferent electrode was a similar pipette with a 50 to 500 μ tip filled with the same solution as the stimulating solution or in some cases 0.1 M NaCl which is what we used for a physiological saline solution. This pipette was inserted into the proximal portion of the detached receptor-bearing part of the fly. In the case of the receptors on the labellum, antennae, and maxillary palpi, the indifferent electrode was inserted into the amputated whole head through the esophageal opening. The wings were mounted by thrusting the indifferent electrode into the proximal end of the costal vein; the ovipositor, by putting the electrode through the abdomen and on into the extended last segment of the ovipositor.

Recording from the tarsal receptors was accomplished in either of two ways. The first was similar to the above with the indifferent electrode inserted into the femur. In the second method an insulated metal microelectrode was inserted into the femur or tibia to make contact with the leg nerve. In this case a Ringer-soaked cotton wick in contact with the pulvillus and a silver chloride–coated silver wire acted as the indifferent electrode. The metal microelectrode was a 20 μ, 70 per cent platinum–30 per cent iridium wire which had previously been sharpened by immersing the tip in a fused salt bath containing a eutectic mixture (70 per cent/30 per cent) of $NaNO_2$; $NaNO_3$ maintained at 290°C. Approximately 1 volt of 60 C.P.S. A.C. was applied between this wire and a passive electrode of the same material. Smooth tapered points less than 1 μ in diameter could be produced in this way. After sharpening, the electrode was coated with General cement polystyrene "Q" dope by dipping and air drying. Platinum black was then deposited on the tip in the usual fashion (1 per cent aqueous solution of choroplatinic acid; wire connected to 6 volts through 11 megohm input vacuum tube volt meter which indicated a good electrode with a steady reading of 5.5 volts or less).

The recording electrode was mounted on a steel spring which could be bent controllably by an electromagnet and cause only a very slight amount of microphonics in the system. Both electrodes were held by Zeiss micromanipulators. These micromanipulators were fastened to a 1 inch steel plate by variable flux permanent magnets in such a way as to minimize vibrations and yet allow complete freedom in locating either micromanipulator.

A high impedance, negative capacitance preamplifier of the MacNichol-Wagner (1954) type was used. The high impedance of this preamplifier made it possible to record using high resistance stimulating solutions such as distilled water or pure sugar solutions as has been described previously (Wolbarsht, 1957). Interference from television and radio was encountered at first and could not be cured by the shielding at our disposal so at the suggestion of Dr. E. F. MacNichol, Jr., we inserted three 3k, ½ watt composition resistors in series from the input grid to the input connector. This method is applicable to any high impedance preamplifier (in a balanced circuit similar strings of resistance would be inserted between each input grid and its input connector). The effectiveness of this resistance in the circuit in suppressing inter-

ference is due to the very low impedance of the tube at high frequencies, which is caused by capacitance and transit time effects. For the much lower frequencies in the desired signal the impedance of the tube is very high so that the effect of the series resistance is negligible. In Fig. 2 we show the response of the amplifier without and with this filter in it. The input tube of this preamplifier, a type 5879, was a specially aged and selected one with a grid current less than 10^{-13} amps. This selection was necessary because preliminary experiments have shown that some preparations are sensitive to currents as small as 10^{-11} amps.

The output of this preamplifier was amplified by both an A.C. and a D.C. system so that small spikes could be examined without annoying base line fluctuations, and slow potentials of large magnitude observed at the same time. Both channels were displayed on conventional oscilloscopes and photographed.

Responses from purely tactile hairs were recorded by amputating the end of the spine and inserting the cut remnant into a pipette containing 0.1 M NaCl which we used to approximate Ringer's solution. Tactile hairs on all parts of the body were studied in this fashion. On the tarsus, in addition to the method described above, recordings were also made by a microelectrode in contact with the leg nerve.

Mechanical deformation of the hair was produced by moving the micromanipulator or by electromagnetically bending the spring on which the recording electrode was mounted. The micromanipulator was used to produce slowly increasing or decreasing large deformations, and the electromagnet produced very small, quick deformations. Since a very steep concentration gradient is produced in the pipette due to evaporation, the tip of the hair was usually inserted as far as possible into the pipette so that motion of the hair and pipette changed the chemical environment at the tip of the hair as little as possible.

In all spike activity observed the recording electrode was initially positive with respect to the indifferent electrode. The only exceptions to this were the recordings made from the leg nerve where the recording electrode's initial deflection was negative. This suggests that the usual recording conditions are analogous to inserting the recording electrode into the cell generating the spike.

RESULTS AND DISCUSSION

Most of the hairs on the fly are purely tactile in function. Recordings made from these hairs are of two types resembling those described by Pumphrey (1936). Some hairs continue responding to a steady deformation; many respond only while moving, the adaptation to any new steady position being over in less than 0.1 sec. Fig. 3 illustrates this type of response recorded in our usual fashion by cutting off the end of the hair. Although this recording technique is quite different from Pumphrey's, it is similar to it as to results. We made a comparison by recording the response from the cut hair through the pipette and then recording the response from the leg nerve through a platinum-iridium electrode. Fig. 4 shows this double recording. The advantage of the first method is the lack of interference from other receptors which may be stimulated at the same time that the hair is moved. These other responses

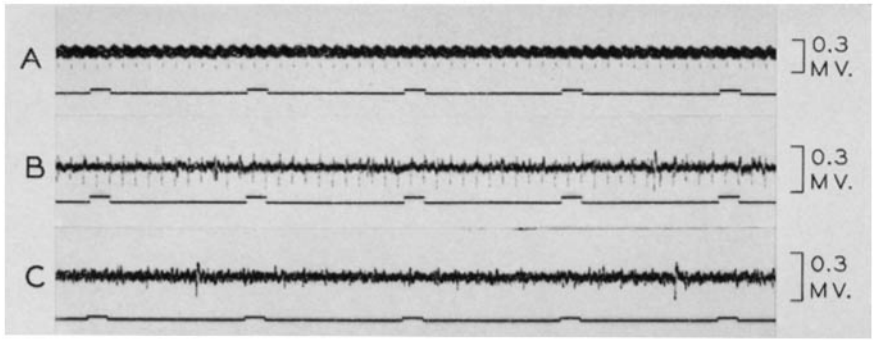

FIG. 2. Recordings from labellar hairs of *Phormia* showing television interference. A, dead hair; television interference, the blanking pulse is easily visible and the video modulation is also present. B, live hair; television interference, the blanking pulse is easily visible as are several nerve impulses from the chemoreceptors. The video modulation cannot be resolved. C, same hair as B. A corrective filter (see text) has been inserted into the amplifier input, which completely eliminates the television interference. 0.01 M NaCl chemical stimulus. Time, 0.2 second. A positive potential at the recording electrode produces a downward deflection on the trace.

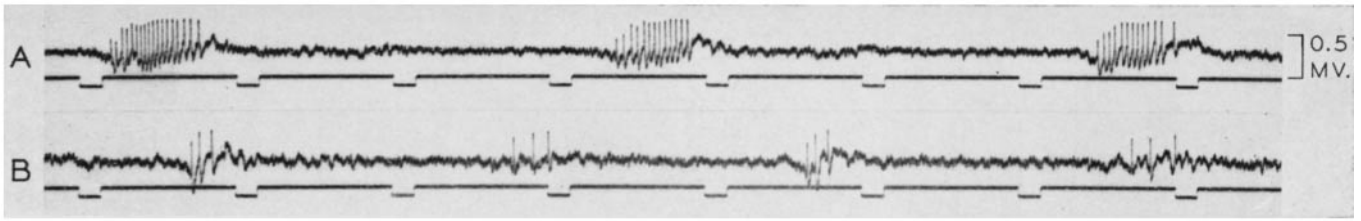

FIG. 3. Response of cut tibial spine to mechanical stimulation, showing adaptation to repetitive stimuli. A, response to motion. B, same hair after 30 seconds of similar stimuli. Time, 0.2 second. Positive potential at recording electrode is up.

are due to slight movements of the joints or vibrations transmitted through the hard cuticle.

An increase in spike height with increase in spike frequency is characteristic of the responses recorded by the method when a pipette is placed in contact with the cut hair. It is probably due to the change in the time course of the impulse and the inadequate high frequency response of the amplifier. A more detailed analysis may reveal the cause of this increase; but whatever the cause, it is still a characteristic of the tactile neuron. This phenomenon is not due to microphonics caused by the motion, for it is found also in the hairs which respond to a steady deformation. Fig. 5 has in the upper trace a response whose initial frequency is 150 to 200 per sec. In the middle record, taken 12 seconds later, the frequency has declined to 40 per sec., with some decrease in spike height. In the lowest record a new deformation increased the frequency to 100 per sec. and also increased spike height.

The tactile hairs responding only to motion adapt to repetitive stimuli. For example, Fig. 3 shows in the upper record the burst of impulses resulting from a rapid motion of the hair. After stimulation in this fashion at the rate of 2 per sec. for 10 to 15 seconds, the pattern of response has changed to that of the lower record.

Although the activity of chemoreceptors could easily be recorded through the stimulating pipette for several hairs simultaneously in the antennae, ovipositor, and maxillary palpi, single neuronal preparations were much less frequent. The chemosensory hairs on the antennae, ovipositor, and maxillary palpi are quite short and very close together. The tip of the stimulating pipette is usually in contact with several hairs at the same time. For this reason it is also difficult to stimulate the same hair with a series of different solutions. Some typical responses are shown in Fig. 6. The lower record is a group of several hairs on the ovipositor, the upper record a single hair from the ovipositor. Similar records were obtained from the maxillary palpi and antennae. The antennal receptors seemed very sensitive to NH_4Cl, even in quite dilute solutions. This is shown in Fig. 7 where the upper record is the response of a single antennal hair to 0.01 M NaCl, and the lower record the response to 0.01 M NH_4Cl. In none of the preparations was a clear-cut response to mechanical stimulation observed. This may be due to the difficulty of applying the proper mechanical stimulus with the recording pipette without at the same time applying a changing chemical stimulus.

Burkhardt and Schneider (1957) have recorded action potentials from the antennal nerve of *Calliphora* caused by mechanical stimulation (air puffs, manipulation of the arista, etc.), but they believe that their responses originated in Johnston's organ and not in the chemosensory hairs. They were unable to record from the nerve any activity caused by chemical stimulation of the chemosensory hairs.

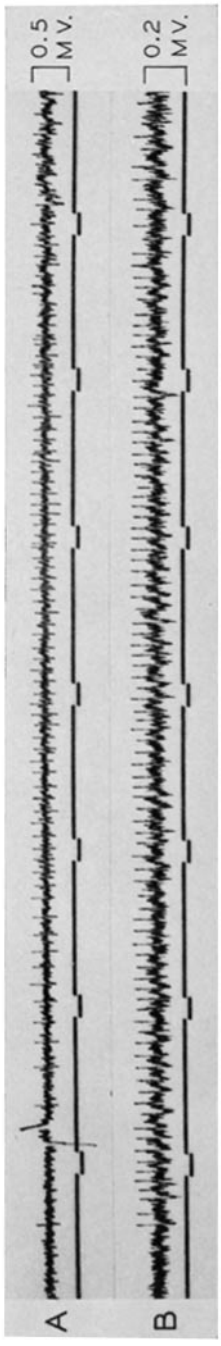

FIG. 4. Response of cut tibial spine to steady mechanical deformation, recorded from the leg nerve and from the cut end of the spine. A, recording from leg nerve. Positive potential at recording electrode is down. B, recording from cut end of spine. Positive potential at recording electrode is up. A and B are responses to similar stimuli administered to the same hair, but were not made simultaneously. Time, 0.2 second.

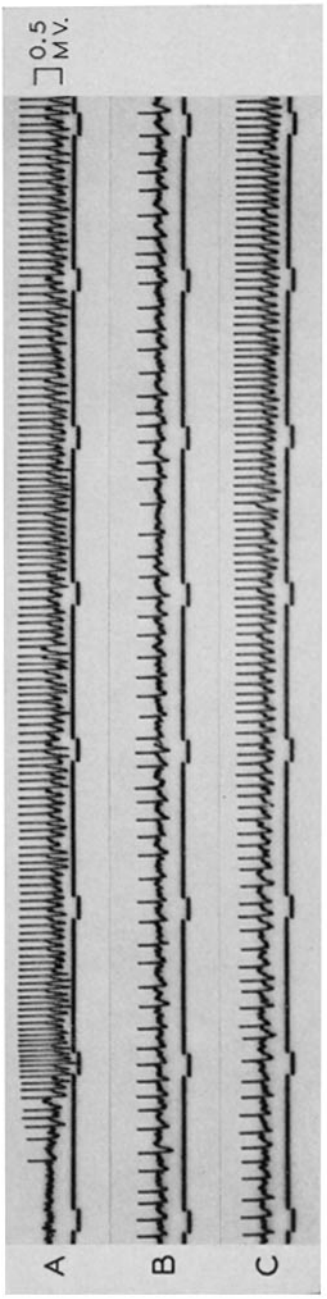

FIG. 5. Response from cut tarsal spine to steady mechanical deformation. These records illustrate the increase in spike height with increase in spike frequency. A, initial deformation. The impulse frequency is 150 to 200/seconds. B, 12 seconds later. The frequency is now 40 per sec. and spike height is quite reduced. C, increased deformation at start of record. Frequency increases to 100 per second. Spike height also increases. Time, 0.2 second. Positive potential at recording electrode is up.

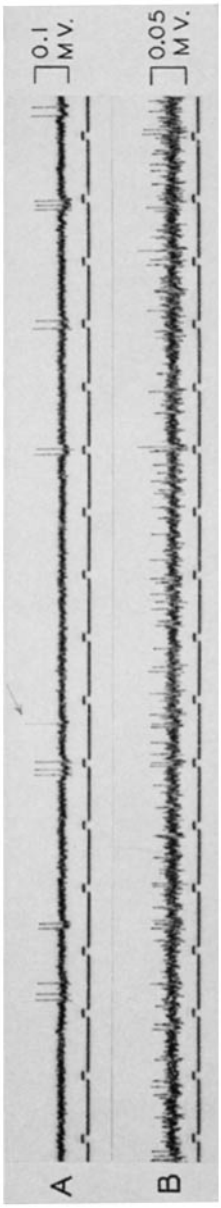

FIG. 6. Response of chemosensory hairs on ovipositor to stimulation by 0.1 M NaCl. A, response of a single neuron. The arrow indicates an electrical artifact. B, response from several hairs at the same time. This is the usual type of record, due to the small size of the hairs and their closeness to each other. Time, 0.2 second. Positive potential at recording electrode is down.

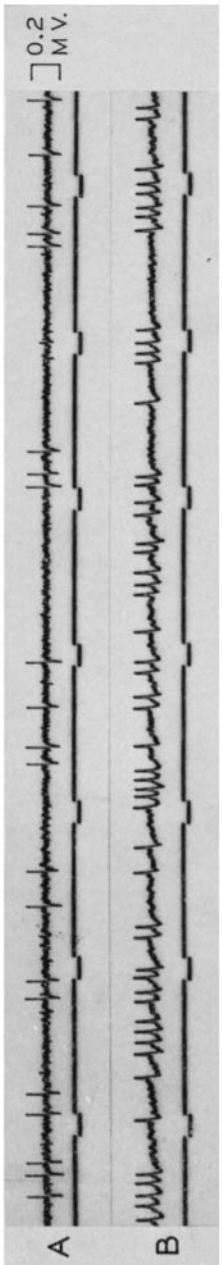

FIG. 7. Response of chemosensory hair on antenna. A, response to 0.01 M NaCl. B, response of same hair to 0.01 M NH_4Cl. The change in spike shape is due to improper adjustment of the capacitance feedback circuit in the amplifier. Time, 0.2 second. Positive potential at the recording electrode is up.

The maxillary palpi have no apparent tactile function in the intact animal although the long spines are innervated and can give spike responses to mechanical stimulation in a manner similar to that of the tactile hairs on other parts of the body. These tactile hairs respond only to motion and not to continued deformation.

The ovipositor is sensitive to touch. This can be correlated with the long tactile hairs that cover it. We have recorded from many of these hairs, all of which are the type that show a continued response to steady deformation. This may aid in positioning the ovipositor for egg laying, since the fly is known to prefer depositing its eggs in cracks and underneath the selected medium. It is unnecessary to ascribe to the chemoreceptors on the maxillary palpi, antennae, and ovipositor any sensitivity to mechanical stimulation. Any behavioral response that has been found to result from gross mechanical stimulation of the above parts can be attributed to the tactile spines.

The electrical response of the labellar hairs to chemical stimulation consists of impulses from two neurons. That there are two neurons can be decided by the occasional electrical adding of the two impulses, and usually, but not always, by the differences in spike size. The larger of the two spikes, in cases in which there is a reliably detectable difference, is stimulated by salts and has been designated the "L spike" (Hodgson, Lettvin, and Roeder, 1955). The smaller of the two impulses, the "S" spike, is evoked mainly by sugars. Both may be stimulated by distilled water (Wolbarsht, 1957).

Both these neurons can be stimulated after the tip of the hair is amputated. The neurons in the cut hair are still differentially sensitive to chemical stimulation, but do not usually respond with as high impulse frequencies as do normal hairs. This lowered frequency may be responsible for the failure of the fly to give a behavioral response when the end of a cut hair is stimulated with an otherwise acceptable solution. This differential response of a cut hair is shown in Fig. 8 in which the three records show the response to alternate applications of 0.01 M NaCl, then 0.01 M D-fructose, which solution produces only S spikes in contrast to the L spikes which are the only ones seen with 0.01 M NaCl. The last record is a return to the 0.01 M NaCl showing that the S spikes were not spontaneous but due rather to the presence of the D-fructose. The cut hair still shows adaptation, and after several minutes the frequency of both L and S spikes in response to either a dilute salt or sugar solution as used above may be as low as one of either spike type every 10 seconds.

Roys (1954) has suggested that a selective filtering action by the cell membranes of the various chemoreceptors is responsible for the differential olfactory sense of the cockroach. He has further suggested that a similar mechanism underlies all differential chemoreception, that wherever the chemoreceptor comes into contact with its environment its membrane is

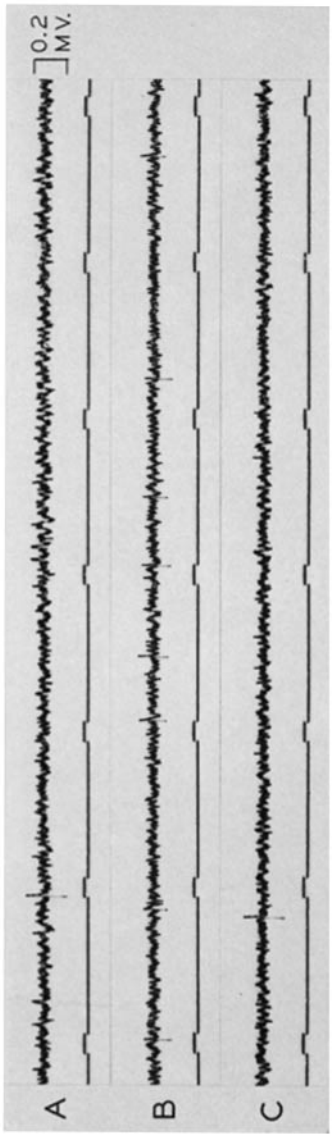

FIG. 8. Differential response by a cut labellar chemosensory hair to various chemical stimuli. A, response to a 0.01 M NaCl. There is only one L type spike response and no S responses. B, response to 0.01 M D-fructose. There are no L type spikes and many S responses. C, response 10 seconds after reapplying 0.01 M NaCl. Note similarity to A. Time, 0.2 second. Positive potential at recording electrode is down.

modified to act as a selective filter for certain chemicals. The differential response of the neurons in the cut labellar hairs of *Phormia* seems to indicate that a selective permeability of each neuron's membrane at the tip of the hair is not the only factor which makes one neuron sensitive to sugar and the other to salt. When the hair is cut, the chemical stimulus may be in direct contact with the cell contents. The drop of liquid that forms after the hair is cut seems to argue against an instantaneous formation of a normal cell membrane over the cut region of the cell (Dethier, 1955). The differential response that occurs at this time may be due to a specialized structure within the cell. There may be an intrinsic difference between the L and S neurons over and above any differential filtering properties possessed by their respective cell membranes.

When a normal, long, labellar hair is bent rapidly by the recording pipette, the activity of a third neuron appears (Wolbarsht and Dethier, 1958). The spike is characteristically small in size, but its size changes with the change in its frequency in the same manner that we have noted previously in purely tactile hairs. This is pictured in Fig. 9 A. The response is evoked only by motion. Steady deformation has no effect after the initial displacement. If the hair is now cut and bent again by the recording electrode, the third neuron can be seen more clearly as in record B. That it is really a third neuron shows in record C, in which the L and S spikes are easily distinguished. At the artifact indicated by the arrow, the hair is bent sharply by an electromagnet. A third spike "M" appears which adds electrically with both L and S. The M spike always has a longer time course than the L and S spikes, as can be seen in this record. The M spike will adapt to continued mechanical stimulation just as the neuron in a tactile hair does.

This agrees quite well with the behavioral responses to similar stimuli which show that the response to mechanical stimuli can be adapted out by several successive applications, leaving the response to chemical stimulation unaffected (Dethier, 1955).

Usually no consistent change is seen in the frequency of the L and S spikes when the hair is stimulated mechanically. This is only true, however, when care is exercised to prevent a concentration change in the solution around the tip. This change may be caused by mixing the relatively concentrated solution at the end of the pipette with the less concentrated solution existing further back in the pipette where the hair tip is, or by moving the hair tip towards the electrode tip. This concentration difference is quite large: crystals of NaCl can form at the pipette tip while the solution 50 to 100 μ inside is 0.01 M NaCl. The effect of this gradient can be demonstrated by noting the increase in spike frequency as the pipette is withdrawn from around the hair, which can be done without disturbing the position of the hair. As the end of the pipette comes close to the tip of the hair, a large increase in the frequency of response is always noted.

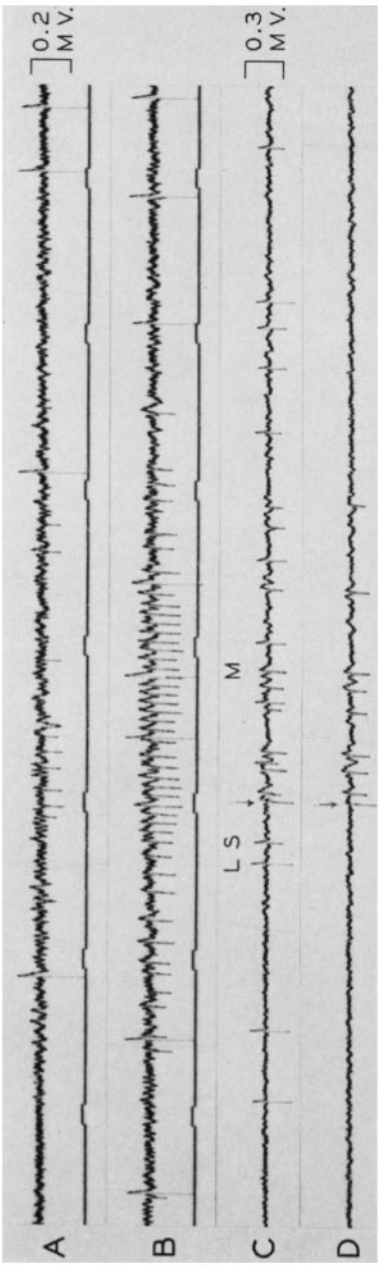

FIG. 9. Response of labellar chemosensory hair to chemical and mechanical stimuli. A, intact hair. 0.01 M NaCl stimulus and motion. M spikes which appear as result of motion are quite small. B, same hair as A, but cut near middle. Same chemical and mechanical stimuli. The L spike is unchanged in size, but the M spike is much larger than in A. C, cut hair. 0.01 M NaCl + 0.1 M D-fructose chemical stimulus. The hair is bent sharply at arrow with change in chemical stimulus (the tip of the hair is moved into the more concentrated solution at the tip of the pipette). D, same as C, except that the chemosensory neurons have become adapted to the solution in the pipette, and the concentration gradient has been removed immediately before the mechanical stimulus so that no simultaneous chemical stimulus is presented. Only M spikes appear as a result of this mechanical stimulus. Time, 0.2 second. Positive potential at recording electrode is down.

Responses due to this cause can be seen in Fig. 9 C. Here, the hair has been inserted into the pipette at an angle such that when the pipette is moved, the hair is bent and the tip of the hair approaches the tip of the pipette. In record D, the same mechanical stimulus is administered. The chemoreceptors have become adapted to their chemical environment and they respond at a very low level with several seconds between responses. The concentration gradient has been eliminated by drawing off some of the solution through the pipette tip immediately before the hair is bent. The response of the mechanoreceptor is identical with that in record C but now no response occurs in either of the two chemosensory neurons. When distilled water is used in the pipette no concentration gradient occurs and consequently only the mechanoreceptor responds, regardless of how the pipette is moved.

Another cause of error can be the random occurrence of both the L and S spikes when the frequency response is low. There are at those times sporadic waxings and wanings of the spike frequency apparently without cause. Since a sudden motion of the hair may coincide with a naturally occurring rise or drop in frequency, an apparent response to mechanical stimulation is seen. This type of response can be recognized and discounted by its lack of reproducibility.

The tarsal chemoreceptors respond very much as do the labellar hairs, with two chemosensory neurons and a third neuron acting as a mechanoreceptor. Grabowski and Dethier (1954) have classified the tarsal chemosensory hairs into four groups on the basis of differences in length. The longest hairs—their type "D"—gave no behavioral response to chemical stimulation. Recordings from the type D hairs were the same as from the other three groups. This indicates that the type D hairs are indeed chemoreceptors, as was suggested by Grabowski and Dethier (1954) on anatomical considerations. The lack of behavioral response to stimulation of a type D hair may be due only to a lower sensitivity of this type of hair. It might also be said at this point that behavioral responses to stimulation of single hairs regardless of location are always quite difficult to obtain reliably.

As was surmised from their structure, the hairs on the wings show electrical signs of chemoreceptor-like activity. The responses are not quite the same, however, as those of the labellar and tarsal hairs. The wing hairs do respond differentially to chemical stimulation; for example, Fig. 10 shows a hair that was stimulated by a mixture of salt and sugar and the upper trace shows the response—a low frequency spike discharge. Stimulation of the same hair with salt alone gives no spike activity at all as is shown in the middle record. A representation of the salt-sugar mixture gives the response in the lowest record which is quite similar to the initial response showing that the hair is still functional. However, hairs can be found which have

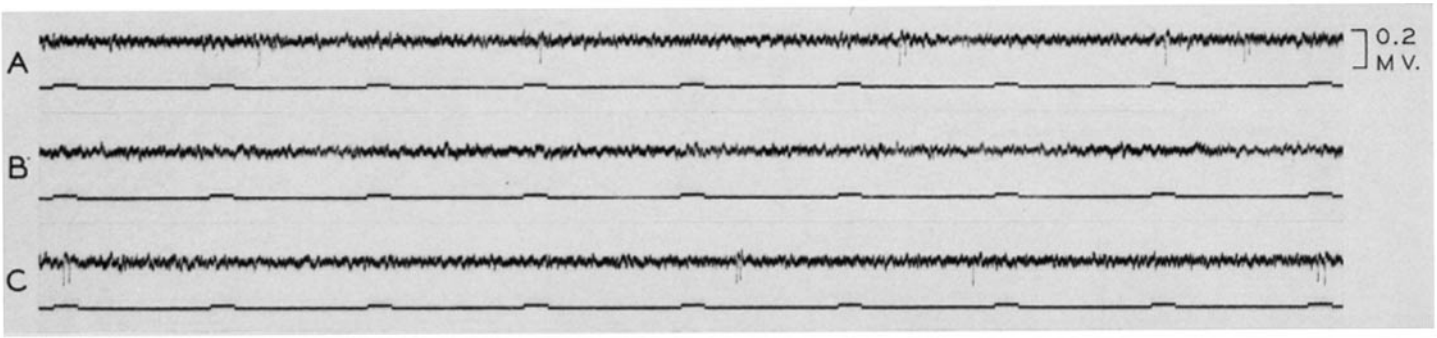

FIG. 10. Response of a wing chemosensory hair to chemical stimulation. A, response to 0.01 M NaCl + 0.01 M D-fructose. B, response to 0.01 M NaCl. Note complete absence of spike activity. C, reapplication of 0.01 M NaCl + 0.01 M D-fructose. Time, 0.2 second. Positive potential at recording electrode is down.

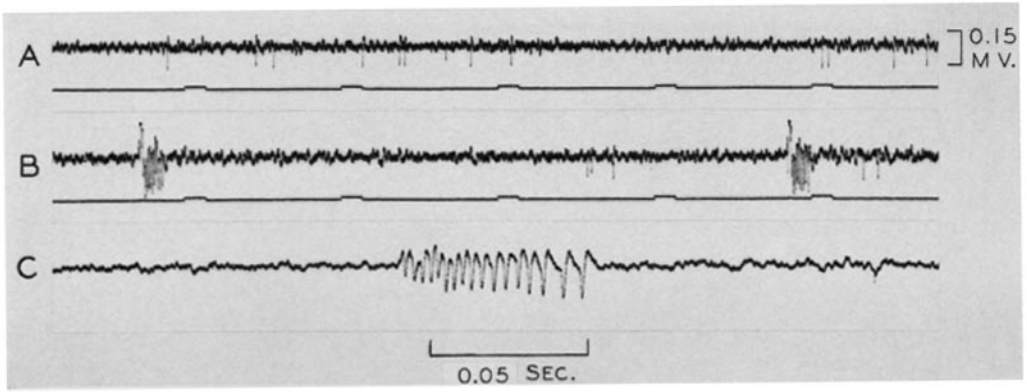

FIG. 11. Response of wing chemosensory hair to chemical and mechanical stimuli. A, response to 0.01 M NaCl. B, response to 0.01 M NaCl and motion. Showing very high frequency bursts of impulses resulting from motion. C, response to motion similar to one of those in B. The time axis has been magnified four times. The maximum impulse frequency here is about 450 per sec. Time, A and B, 0.2 second. C as indicated. Positive potential at recording electrode is down.

the opposite type of response. That is, hairs which respond well to a salt solution, do not respond to a salt-sugar mixture, and which will still respond when stimulated again by the original salt solution have also been found. No behavioral responses have been demonstrated as yet from the application of various salt and/or sugar solutions to these hairs, and the function of these hairs as chemoreceptors is at the moment somewhat mysterious.

The response of the wing neurons to mechanical stimulation is very vigorous. This is shown in Fig. 11 which has in A the chemoreceptor responses, then in B the response to deformation of the hair. The response occurs only while the hair is being moved and consists of a burst of impulses at very high frequency. In C one of these motion responses is shown with the time axis magnified four times. This allows the individual impulses to be seen clearly. In this record the spikes have an upper frequency of about 450 per sec. Responses have been obtained which have a frequency of 600 or more per sec.

The variation of spike height with frequency in the wing neurons is not quite the same as in the mechanoreceptor neurons of the chemosensory hairs of the labellum and tarsus. In the wing neurons no constant relation is seen; sometimes the largest spikes have the lowest frequency as shown in Fig. 11 C. At other times the spike size is constant in spite of changes in frequency. Indeed, random variations of size within a single burst of impulses have been seen, as shown in the middle record of the same figure. This change in spike size seems to be correlated more with fluctuations in the base line than anything else and could even be caused by microphonics in the electrode. Microphonics could play a large role in these recordings because of the small size of the spikes and the great amplification required.

Only a small displacement of the hair is required to produce a mechanoresponse if the motion is parallel to the surface of the wing and towards the trailing edge of the wing. The speed of the displacing motion is the important parameter. Slow movements have no effect at all. The more rapid the motion, the more vigorous the response. It is possible that a puff of air moving over the surface of the wing would bend the hair enough to cause a response. The hair would be most sensitive to stimulation by air moving from the front to the back of the wing. Since this is the normal motion of the air during the power part of the wing beat, the hair would give its largest response at this time.

The normal wing beat frequency of this species is about 200 per sec. (Hudson, 1958). Thus these receptors could easily respond if stimulated on both the up and down strokes of each wing beat. Pringle (1957) has suggested that these receptors would not be able to mediate the rapid reflex actions necessary for the control of flight. The proximal nerve fibers of these neurons have a very small diameter and a correspondingly low impuse conduction velocity. This low conduction velocity would cause a large interval between

the time when the hair is stimulated and the time that the central nervous system could use this information to adjust the shape and attack angle of the wing. This interval would prevent any change in wing shape or attack angle during a wing beat as a result of information received during the same wing beat. However, it is quite possible that information received during one wing beat can be used as the basis for altering the wing shape and attack angle several wing beats later.

To develop this idea further the motion of the wings in normal flight must be considered. The up and down motion is automatic (Pringle, 1957). Once it is initiated it continues at a relatively constant rate and amplitude more or less independently of any nervous control by the fly, until it is stopped completely. The fly can control the shape and attack angle of its wings during the various parts of the stroke, and in this way is able to develop lift and move in the direction it wishes.

The rapidity of the wing beat suggests that each single wing stroke contributes very little to the motion of the fly. If the wing mechanoreceptors can detect the motion of the air over both the upper and lower surfaces of the wing, then they would be able to tell when the attack angle of the wing was larger than the stalling angle. Under these latter conditions the air would be moving smoothly over the lower surface of the wing but not over the upper surface of the wing. This is opposed to the equal air flow and consequently equal stimulation of the mechanoreceptors on the upper and lower surfaces during a wing stroke in which the stalling angle is not exceeded by the attack angle and consequently some lift is developed. As the attack angle approaches the stalling angle, the stimulation of the mechanoreceptors on the upper surface would be decreased, and before many wing beats had passed the attack angle would be altered by the fly enough to give a more nearly equal stimulation of the two sets of mechanoreceptors, thereby avoiding a stall.

The fly has a thin wing which develops its maximum lift at an attack angle very close to the stalling angle. The wing hairs would aid in flight efficiency, allow the wing to operate close to the stalling angle, and thus develop maximum lift by removing the danger of stalling. Efficient flight probably requires a constant series of corrections of the attack angle based on information from these hairs.

Besides controlling the attack angle, the fly can control the camber of different areas of the wing. These mechanoreceptors may aid the fly in getting the best over-all shape for any particular attack angle. In this case, if the camber were too large no air would move over the lower surface of the wing and this could be detected by the mechanoreceptors.

We might mention here that in insects which have larger wings, or wings capable of being folded into more complicated patterns during flight, hairs similar to those on the fly wing may be found not only on the leading edge,

but also on other parts of the wing (Zácwilichowski, 1933). The hairs may have, in these cases, a similar function in aiding the insect in shaping his wings to yield the highest efficiency for a particular set of circumstances.

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