Central projections of labellar taste hairs in the blowfly *Phormia regina* Meigen and their positional effects on proboscis extension

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SHORT TITLE

Central projections of taste hairs in the blowfly

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GENERAL ABSTRACT

The positional information of a stimulus encoded by a group of labellar gustatory sensilla, the "largest" hairs, was studied. There are 11 largest hairs arrayed along each labellar lobe. Each hair contains five sensory cells. Four cells respond to chemical stimulation; two to salt, one to sugar and one to water and the fifth cell is a mechanoreceptor. Individual or pairs of hairs were stimulated with sugar or water to elicit a proboscis extension. The direction of proboscis extension is graded according to the position on the lobe of the hair(s) stimulated. Hairs situated on the anterior region of the lobe elicit anterior extensions, posterior hairs elicit posterior extensions and mid-level hairs give lateral extensions.

The central morphology of cells contained in the largest hairs was studied to determine whether the individuality of each hair observed in the behavioral experiments is reflected in the field of terminal arborization formed by the cells innervating it. There was no continuous somatotopic representation of the hairs. Instead, the central morphology of the 11 hairs fell into three groups. Anterior hairs 1 and 2, mid-level hairs 3 through 8 and posterior hairs 9 through 11 each formed a distinct central branching pattern in the sub-esophageal

ganglion.

RESUME GENERALE

La possibilité qu'un groupe de sensilles gustatives des labelles, nommées les "plus grands" poils, encode la position d'un stimulus a été étudié. Chaque lobe des labelles porte 11 poils "plus grands" disposés regulièrement sur sa surface. Chaque poil contient cinq cellules sensorielles. Quatre possèdent une sensibilité chimique; deux au sel, une au sucre et une à l'eau et la cinquième cellule est mecanoreceptrice. Les poils ont été stimulés individuellement ou par pairs avec une solution sucrée ou de l'eau pour obtenir une extension de la trompe. La direction de l'extension de la trompe est dépendante de l'identité du poil stimulé. La direction de la reponse reflète la position du poil sur le lobe. Les poils situés sur la region antérieure du lobe engendrent des extensions plutôt antérieures, ceux situés postérieurement engendrent des extensions postérieures et les poils à mi-niveaux produisent des extensions latérales.

La morphologie centrale des cellules qui innervent les poils a été etudiée pour déterminer si la morphologie de l'arborization terminale de chaque poil reflète l'individualité observée dans l'étude du comportement. La morphologie centrale de chaque poil n'est pas distincte, les poils sont plutôt groupés en 3 classes. Les poils un et

deux, les poils trois à huit et les poils neuf à onze ont chacun une différente morphologie centrale.

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PREFACE

In accordance with the options offered by the faculty of Graduate Studies and Research, this thesis is composed of two papers. One paper is in press in the journal Cell Tissue Research and the other has been submitted to The Journal of Comparative Physiology. In both papers I am the first author (and my thesis supervisor Gerald Pollack second author) and so am primarily responsible for their content and the research which they involved.

ACKNOWLEDGEMENTS

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GENERAL INTRODUCTION

To understand the neural basis of a behavior, one needs first to comprehend the physiology and mechanics of the various components of the behavior and then relate them to the physiology and anatomy of neuronal cells which mediate the behavior.

The feeding behavior of the fly is a good model for the study of principles in neural circuitry. The small size of the insect, the reliability with which the behavior can be induced to occur and the ease with which specific components of its environment can be manipulated are but a few examples of why this system has many times been chosen as a model. Much is known about the mechanisms which gate the various components of the behavior. The fly approaches a food source when attractive volatile components of that source stimulate its olfactory receptors. Subsequent components of the behavior are controlled by three sets of external gustatory receptors. For example, when chemoreceptors contained in tarsal sensilla contact food the proboscis is extended out of the head capsule bringing its terminal segment, the labellum, in contact with the food. The paired labellar lobes carry aboral and oral gustatory sensilla. Receptors contained in these are responsible for spreading the labellar lobes (Pollack, 1977) and triggering imbibition

(Dethier, 1976), respectively. Although under natural conditions chemosensilla present on the labellum come in contact with food only when the proboscis has been extended, usually by stimulation of the tarsi, a fly can be induced to extend its proboscis by directly stimulating chemosensilla on its labellum (Minnich, 1931). Stimulation of single "largest" hairs, a group of 11 labellar sensilla arranged sequentially on each lobe (Wilczek, 1967), can induce a full extension of the proboscis.

Whether or not the fly extends its proboscis upon stimulation depends on its physiological state. Hungry flies and water deprived flies will more readily extend their proboscis to sugar (Evans and Dethier, 1957; Evans and Browne, 1960) and to water (Dethier, 1961) stimulation, respectively. Lower concentrations of sugar and adulterated water will be accepted with increasing deprivation periods. The strength of activity of the primary receptor cells however does not vary with the fly's physiological state. Their activity is directly proportional to the concentration of the salt and sugar solutions for the salt (Evans and Mellon, 1962a) and sugar (Omand and Dethier, 1969) cells respectively, and inversely so for the water cell (Evans and Mellon, 1962b) suggesting that an internal satiety mechanism, and not peripheral activity, sets the receptivity level of the fly. Getting (1971) has shown that indeed the level of primary receptor activity required to elicit a motor response decreases with increasing starvation

period. The frequency of initial sensory activity required to elicit a motor response was 2 times greater for flies starved between 62 to 66 hours than for flies starved 70 to 72 hours.

Several muscles are involved in the extension and steering of the three segments of the proboscis. The most proximal segment, the rostrum, is extended out of the head capsule by contraction of the paired fulcri (rostral) protractors (Van der Starre, 1977; Thomson, 1977). Four other sets of muscles control the extension and lateral movements of the haustellum and labellum (Dethier, 1959). These five sets of muscle allow for considerable movement control of the segments of the proboscis. Yetman and Pollack (1986a) (Chapter 1, this volume), have shown that the proboscis can indeed be extended over a wide range of angles by stimulating individual or pairs of largest hairs. The directional component is dependent on the identity of the hair stimulated. They have suggested that individual hairs, through different interactions with second order neurons, activate the muscles of the proboscis differentially so that anterior hairs, for example, drive the proboscis anteriorly, lateral hairs cause rather skewed lateral extensions and so on.

Recently Van Mier et al. (1985) have traced the dendritic fields of motor neurons innervating muscles which

control the extension of the proboscis. These cells branch extensively in the sub-esophageal ganglion. Yetman and Pollack (1986b) (Chapter 2, this volume) have demonstrated that the synaptic field of receptors contained in the largest hairs is also restricted to the sub-esophageal ganglion. It is therefore possible that the receptors be connected directly or through single interneurones which lie within the sub-esophageal ganglion. The anatomical investigations have provided information about the probable location of as yet undiscovered links in the pathway. It is now possible to search for such links within the neuropile of the sub-esophageal ganglion.

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CHAPTER I

Proboscis extension in the blowfly: directional responses to stimulation of identified chemosensitive hairs

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ABSTRACT

We studied the direction of proboscis extension elicited by stimulating each of an identified array of gustatory sensilla, the "largest" hairs, on the labellum of the fly *Phormia regina*. Individual hairs, or pairs of hairs, were stimulated with sucrose or water and the angle of the ensuing extension of the proboscis was recorded. The direction of the response was graded and depended on the identities of the hairs stimulated. Hairs situated on the anterior region of the labellum elicited anterior extensions, mid-level hairs elicited lateral extensions and posterior hairs resulted in posterior extensions. Previous studies of the labellar hairs have been concerned with their encoding of the chemical nature of the stimulus. Our findings show that each hair also relays information about its location. The positional information provided by these sensilla may help the fly to orient itself with respect to a food source.

INTRODUCTION

The first step in the fly's feeding response, extension of the proboscis, occurs when chemoreceptors on its tarsi encounter an acceptable substance as the fly wanders on a substrate. Proboscis extension brings another set of sense organs into play, namely the taste hairs situated on the aboral surface of each labellar lobe. Appropriate stimulation of the chemoreceptors housed in these hairs causes the labellar lobes to spread apart so that yet another group of receptors, which are situated on the oral surface, can sample the food and initiate imbibition (see Dethier 1976 for review). This sequence of movements is oriented in space with respect to the location of the food source. For example, flies reliably turn in the direction of the stimulated leg before extending the proboscis (Dethier, 1976). Similarly, stimulation of the labellar taste hairs on only one side can result in asymmetric lobe-spreading, with the result that the oral surface is appropriately directed toward the food source (Pollack, 1977). Although a great deal of attention has been focused on understanding how the fly's taste organs provide it with information about the quality of a potential food source, relatively little work has examined how the fly determines the location of the food source. This is the subject of the present report.

Dethier (1976) showed that a fly is able to follow a narrow streak of sucrose solution using information obtained from receptors on the proboscis. We have investigated the extent to which a particular group of taste sensilla, the "largest" labellar hairs, carry information about the location of the stimulus. Although labellar hairs do not normally come in contact with food until after the proboscis has been extended, it has long been known that when tarsal stimulation is bypassed stimulation of the labellar hairs can elicit proboscis extension (Minnich 1931). In the present paper we ask whether the direction of proboscis extension depends on which of the largest labellar hairs is stimulated. Each of the paired labellar lobes carries approximately 125 taste hairs. These have been divided, on morphological grounds, into six classes (Wilczek, 1967), one of which is composed of the 11 largest hairs. Each largest hair houses four chemoreceptors (so-called sugar, water, salt and anion receptors, respectively) and one mechanoreceptor (Peters and Richter, 1965; Dethier, 1976). In chemosensory function the hairs are similar. They differ, however, in their positions on the labellum, being arranged longitudinally with hair 1 most anterior and hair 11 most posterior (Wilczek, 1967). This spatial arrangement allows the possibility that the position of a stimulus, and thus the direction of the response it elicits, could be derived by determining which hairs were stimulated. We find that the hairs do encode the location of the stimulus; the

direction of proboscis extension varies, in an orderly way, with the identity of the hair that elicits the response.

MATERIALS AND METHODS

Phorsia regina (Meigen) were cultured in the laboratory. Larvae were reared on an artificial medium (Hill, et al. 1947) at 29° C. Adults were kept at 29° C from the time of emergence until testing. All experiments were done at room temperature (21-24° C).

STIMULATION WITH SUCROSE

Preparations

Newly hatched flies were given sucrose and water ad libitum for 1 to 12 days. Flies were then allowed only water for 3-5 days. After starvation, flies were anaesthetized by chilling and were immobilized, ventral side uppermost, by fixing their wings, legs and head to a block of wax.

Experimental procedure

With the labellum in a retracted position, a single largest hair was stimulated by gently touching its tip with a small drop of 1.0M sucrose solution extruded from the tip of a micropipet. The droplet was kept in contact with the hair for a maximum of five seconds or until a response occurred. To minimize variability caused by evaporation, a new drop was extruded prior to each test. A particular hair

of a given fly was tested only once (except when extension was less than complete; see below). Ten minutes elapsed between stimulation of different hairs of the same fly. Stimulation sometimes elicited only partial extension of the proboscis. The degree of extension was measured according to a scale devised by Dethier et al. (1963). This numerical scale ranges from one to six, with position one representing very slight extension of the labellum out of the oral cavity, position three extension of the haustellum and part of the rostrum, and position six full extension of the haustellum and rostrum. If the extension elicited by stimulating a particular hair was only to position two or less, the same hair was restimulated immediately, up to three times in succession. If a more vigorous response was not produced by this series of stimuli then no data were collected for that hair from that fly. To determine if stimulating the hair more than once had an effect on the resultant direction of extension, we compared the direction obtained after single stimulation with that obtained after repeated stimulation for each of the 11 largest hairs. A significant difference was found only for hair 3 (Mann-Whitney U-test, $p=.03$). The responses to single and repeated stimuli were therefore pooled in all statistical analyses. To test the possibility that the flies obtained spatial information about the stimulus from visual or olfactory cues, several experiments were performed with the antennae and eyes of the flies covered with wax. Eliminating visual and olfactory input in this way did not affect the

directionality of the responses (Mann-Whitney U-test, $p>.05$ for all hairs). The results from flies with unobstructed and waxed eyes and antennae were therefore also pooled.

Measurement of direction

The fly was placed under a dissecting microscope and positioned so that the middle of its labellum was centered in the field of view. A grid in the eyepiece of the microscope divided the field into 12 sectors of 30° each. Two general types of extension were observed. In some cases the labellum moved almost exclusively in the vertical plane, upwards towards the experimenter, and stayed in the center of the field of view. In these cases (when the labellum remained within a radius of approximately 0.6 mm from the center of the field of view) the responses were recorded as "upward". The freqency of upward responses was greatest for hair 1 (20%), and was less than 10% for all other hairs. Upward responses are not considered further in this study. In the majority of cases the extension of the proboscis had a strong horizontal component, which brought the labellum more than 0.6 mm, and often 1.5 mm, away from the center of the field of view. In these cases the horizontal component of the direction of extension was estimated to the nearest 15° using the grid in the eyepiece (Fig. 1).

STIMULATION WITH WATER

Preparations

Newly emerged flies were allowed free access to 0.25M sucrose for one or two days. They were then deprived of both water and sugar for an additional two days. The flies were prepared for experiments as described above except that the tarsi were kept free of wax for future stimulation (see below). To prevent the legs from interfering with labellar stimulation they were restrained at the tibial-femoral joint with miniature staples.

Experimental procedure

We found that stimulation of single hairs, or even of two adjacent hairs, with water only rarely elicited proboscis extension. In order to study the response to water more readily, we increased the central excitatory state (CES) of flies before applying the water stimulus. Dethier et al. (1965) showed that stimulating a fly with an attractive stimulus (sucrose) increases its central excitatory state, thereby increasing the probability of response to subsequent stimulation. Since the flies did not respond to stimulation of one or two hairs with water, we increased their CES a few seconds before each water stimulus by stimulating the tarsi with sucrose. All six tarsi were dipped simultaneously in increasing concentrations of sucrose (0.063, 0.125, 0.25 and 0.5M) until the proboscis was extended. Flies that did not respond to any of these sucrose concentrations were discarded. Once a response occurred, we waited until the proboscis was retracted again

(usually within 10 seconds) and then stimulated two neighboring largest hairs at the same time with a large drop of water (stimulating only a single hair did not reliably elicit extension even after increasing the CES).

ANALYSIS OF RESULTS

The results were analyzed with circular statistical procedures (Batschelet, 1981). Two parameters were computed for each population of responses, the mean direction of extension and an index of the variability of the response, the r value. r approaches 1.0 when the responses are clustered and decreases, to a minimum of 0, with increasing scatter in the data. The results are plotted as polar diagrams. The mean direction of extension is represented by the direction of an arrow and the r value is represented by the arrow's length (an arrow equal in length to the radius of the diagram corresponds to an r value of 1.0). The diagrams were constructed as if all stimuli were applied to left hairs (which was true in the majority of the experiments). Responses to stimulation of right hairs were adjusted by reflecting the angles of extension across the midline.

RESULTS

Stimulation with sucrose

The distributions of the directions of proboscis extension for an anterior hair (hair 2), a mid-level hair (hair 6) and a posterior hair (hair 10) are shown in Fig. 2. It is clear that, for each of the hairs, the responses are clustered around a particular direction, which is hair-specific. Fig. 3 summarizes response directions for all eleven largest hairs. It can be seen that the direction changes, in an orderly and continuous manner, according to the identity of the stimulated hair; anterior hairs elicit anteriorly-directed extensions, mid-level hairs elicit lateral extensions, and posterior hairs elicit posteriorly-directed extensions. Thus, the sensory message generated by stimulating a single largest labellar hair with sucrose includes information about the identity of the hair, and therefore about the location of the stimulus.

Stimulation with water

Stimulating a single hair, or even two adjacent hairs, of a thirsty fly with water did not reliably elicit extension of the proboscis. However, when the central excitatory state (CES) was increased just before labellar stimulation with water, simultaneous stimulation of two adjacent hairs did consistently elicit responses (see

Materials and Methods). To determine whether this method of stimulation obscures the directionality of proboscis extension, we stimulated pairs of hairs in CEB-enhanced flies with 1.0M sucrose.

Fig. 4 illustrates the results of this control experiment. The mean direction of extension for the five most anterior pairs of hairs (1+2 through 5+6) varied between 96° (hairs 4+5) and 131° (hairs 5+6), but with no apparent correlaton with hair position. For the posterior pairs of hairs (6+7 through 10+11), the mean directions of the responses were graded according to the hairs' positions.

Many of the response distributions for the anterior pairs appear to be bimodal. This is most evident for the pairs 1+2, 2+3 and 3+4 as indicated in Fig. 4. Inspection of these distributions suggests that the anterior, but not the posterior responses may vary systematically with the identities of the hairs stimulated. If this were true then the unimodal distributions of responses seen for more posterior pairs of hairs might be explained by the greater coincidence between the angle of extension determined by the identities of the stimulated hairs and the angle typical of hair-independent responses. In order to investigate the possibility that the responses to simultaneous stimulation of anterior pairs of hairs fell into two classes we divided the responses for each of these pairs into two groups, those with extension angles clockwise of 308°, up to 127° (the

presumed hair-dependent group) and those with extension angles clockwise of 128°, up to 307° (the presumed hair-independent group). These limits were chosen both on the basis of the distributions of the responses to the first three pairs of hairs and because they include the mean directions of extension for single-hair stimulation of hairs 1 through 6. The effect of separating the responses in this way is shown in Fig. 5. The two subgroups of responses are demarcated by a dashed line. The means for the responses that are presumed to vary with hair identity are indicated by dashed arrows, and those that are presumed not to vary systematically with hair identity are indicated by solid arrows. Responses in the latter subgroup differ significantly among the pairs of hairs ($p < 0.03$, Kruskal-Wallis test), but there is no systematic relationship between the direction of extension and the positions of the hairs that were stimulated. In contrast, the mean directions of extension for the presumed hair-dependent subgroup of responses (which also differ significantly from one another; $p < 0.03$) do vary systematically with hair position, ranging from 56° for hairs 1+2 to 102° for hairs 5+6.

Fig. 6 summarizes the response directions for the ten pairs of hairs. The means for the anterior 5 pairs were calculated only from the responses that lie clockwise of 308°, up to 127°, while those for the posterior pairs

include all responses. It can be seen that, as for single-hair stimulation, the direction of extension varies in an orderly manner with the identities of the stimulated hairs.

We used the same dual-hair paradigm to test whether information about the location of a water stimulus is also provided by labellar hairs. Fig. 7 shows responses to stimulation of hairs 2+3, 6+7 and 10+11. The distributions of responses were similar to those just described for sucrose. Responses to stimulation of posterior pairs of hairs were unimodally distributed while responses to anterior pairs appeared to be bimodally distributed (Fig. 7, hairs 2+3). The responses for these anterior hairs (pairs 1+2 through 5+6) were divided into the same two sub-groups described above.

Fig. 8 summarizes responses to water. As in Fig. 6, the dashed arrrows for the anterior pairs of hairs represent means computed from responses that fell between 308° and 127°. It can be seen that, with the exception of the pair 1+2, response direction is continuously graded according to hair position. Thus, the largest labellar hairs provide information about the location of a water stimlus as well as of a sucrose stimulus.

DISCUSSION

Localization of a chemical stimulus with a receptor array

There are two well-documented general strategies that animals use to obtain spatial information about a chemical stimulus (Bell and Tobin, 1982). One is to move through the environment and compare samples taken in different places at different times (klinotaxis: Havukkala, 1980; Bell and Tobin, 1981) and the other is to compare the activity of spatially separated receptors which simultaneously sample different regions of the environment (tropotaxis: Martin, 1964; Bell and Tobin, 1981). We have demonstrated that flies use a third strategy; they derive spatial information about a taste stimulus by determining which receptors within a spatial array have been stimulated. Such a point-to-point mapping between receptor arrays and oriented behaviors is well-known in visual responses (Sperry, 1951) and somatosensory responses (Levine et al. 1985; Cahmi, 1974), but it has only rarely been implicated in chemosensory responses. A chemosensory example has recently been reported by Morita and Finger (1985). They described the orderly projections into the central nervous system of taste receptors that are spatially arrayed in the goldfish's mouth, and suggested that they might be involved in the fish's selection of food items for ingestion from different regions of the mouth.

The labellar hair is not an exclusively chemosensory sensillum; it also houses a mechanoreceptor. It is possible, therefore, that the spatial information revealed in our experiments was provided not by chemoreceptors but by mechanoreceptors. We feel this is unlikely. Our stimuli were applied gently, without noticeably bending or displacing the hairs. Unpublished electrophysiological experiments, in which we recorded from taste hairs under a variety of stimulus conditions, suggest that the mechanoreceptor would not have been activated by our stimuli. It is possible, however, that the mechanoreceptor might have been stimulated by renewed contact between labellar hairs and the stimulating pipet as the proboscis began to extend. Although we cannot rule out this possibility for the majority of our experiments, sometimes the proboscis was extended slowly enough that the stimulating pipet could be removed in time to avoid with certainty renewed contact with the hairs. The directions of these slow responses depended on hair identity in a manner similar to that described for the responses as a whole. Furthermore, it is possible to detect hair-dependent differences in the initial components of the proboscis extension. When posterior hairs are stimulated, for example, the haustellum, a distal segment of the proboscis, usually unfolds first from the more proximal rostrum, which only later is extended from the head capsule, whereas when anterior hairs are stimulated extension of the rostrum

occurs shortly before or at the same time as extension of the haustellum. This suggests that the direction of the extension is, at least in part, predetermined at the onset of the response, probably by the activity of chemosensory cells.

Directional extension of the proboscis was obtained both with sucrose and water stimuli. Water stimulates the water receptor exclusively (Evans and Mellon, 1962), whereas sucrose solutions can stimulate both the sugar and water receptors, with the degree of excitation of the water receptor inversely proportional to the sucrose concentration. At the concentration used in our experiments (1.0M), activity of the water receptor is substantially depressed (Rees, 1970). It is possible, therefore, that both the sugar and water receptors carry information about stimulus location.

Effect of tarsal stimulation on direction of extension

Stimulating labellar hairs with water or sucrose after the CES was raised by tarsal stimulation with sucrose elicited a large number of posterior extensions which did not vary systematically with hair identity. In some cases, the fly appeared to be attempting to bring its labellum and tarsi in contact. For several seconds following proboscis extension, the labellum moved in a probing manner and the prothoracic tarsus ipsilateral to the stimulated labellar

hairs was bent towards the head. The proboscis was only rarely extended towards the tarsus of the unstimulated side, suggesting that even in these apparently non-hair-specific extensions the labellar hairs controlled some component of the direction of the response. These results also show, however, that the direction of proboscis extension is not exclusively determined by the labellar hairs, because tarsal stimulation also has an effect. Stimulation of labellar hairs in these experiments often followed tarsal stimulation by several seconds. The tarsal effect on directionality was, therefore, a long-lasting one. Its long duration is possibly due to continued chemosensory input provided by residual sugar on the tarsi.

Anatomical projections of taste receptors, connectivity, and behavior

The finding that the direction of proboscis extension varies according to the identity of the hair(s) which elicit the response implies that sensory neurons from different hairs make hair-specific choices in the selection of, or strengths of interaction with, the second-order cells with which they synapse. Work in other systems (Levine et al. 1985, Bacon and Murphey 1984) has shown that one important factor that determines the connectivity of receptor cells is the region of their termination within the central nervous system. Yetman and Pollack (1986) mapped the central projections of the largest hairs with cobalt staining and found that they fall into three distinct groups, comprising

hairs 1 and 2 , hairs 3 through 8, and hairs 9 through 11, respectively. The projections from the individual hairs within each of these groups are similar. If the region of central termination were the only determinant of connectivity, then it might have been expected that the response directions would fall into three clusters, each corresponding to one of the anatomical classes. Instead, we found a continuous gradation of directions. These results imply that sensory neurons from different hairs can differ in their central connectivity even though they project to the same region of the central nervous system. A similar conclusion was reached by Murphey et al. (1984), who found that mechanoreceptors associated with different sensillum types on the cerci of crickets (clavate hairs and filiform hairs), which code for different modalities (detection of orientation in the gravitational field and of air displacement, respectively), terminate in the same region of the last abdominal ganglion, but nevertheless probably select different postsynaptic targets. In the case of the fly, the sensory neurons in question are associated with the same sensillum type (largest labellar hair) and code for the same modality (either sugar or water).

Role of spatial information in behavior

The behavior that was studied here, extension of the proboscis upon stimulation of labellar taste hairs, does not occur under natural circumstances because the labellar hairs

normally do not come into contact with the stimulus until after the proboscis has been extended. Nevertheless, the encoding of spatial information by these sensilla may be relevant to the natural feeding behavior of flies. One component of this behavior is the "dance". This is a locomotor pattern which is adopted when imbibition of a small amount of food has left the fly unsated. The dance is thought to serve to increase the likelihood of the fly's encountering additional food patches (Dethier 1957; Nelson 1977). During the dance the proboscis is repeatedly extended and retracted, as if the fly is probing the surface with its labellar chemoreceptors. It seems likely that, if this labellar probing is successful in discovering a food source, the spatial information provided by the labellar hairs may be used to orient the fly, or its proboscis, with respect to the stimulus.

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Fig. 1 Measurement of response direction. The fly was viewed through a grid placed in the ocular of a dissecting microscope, and the horizontal component of the response direction was measured to the nearest 15°. The direction of the response illustrated here would be recorded as 75°.

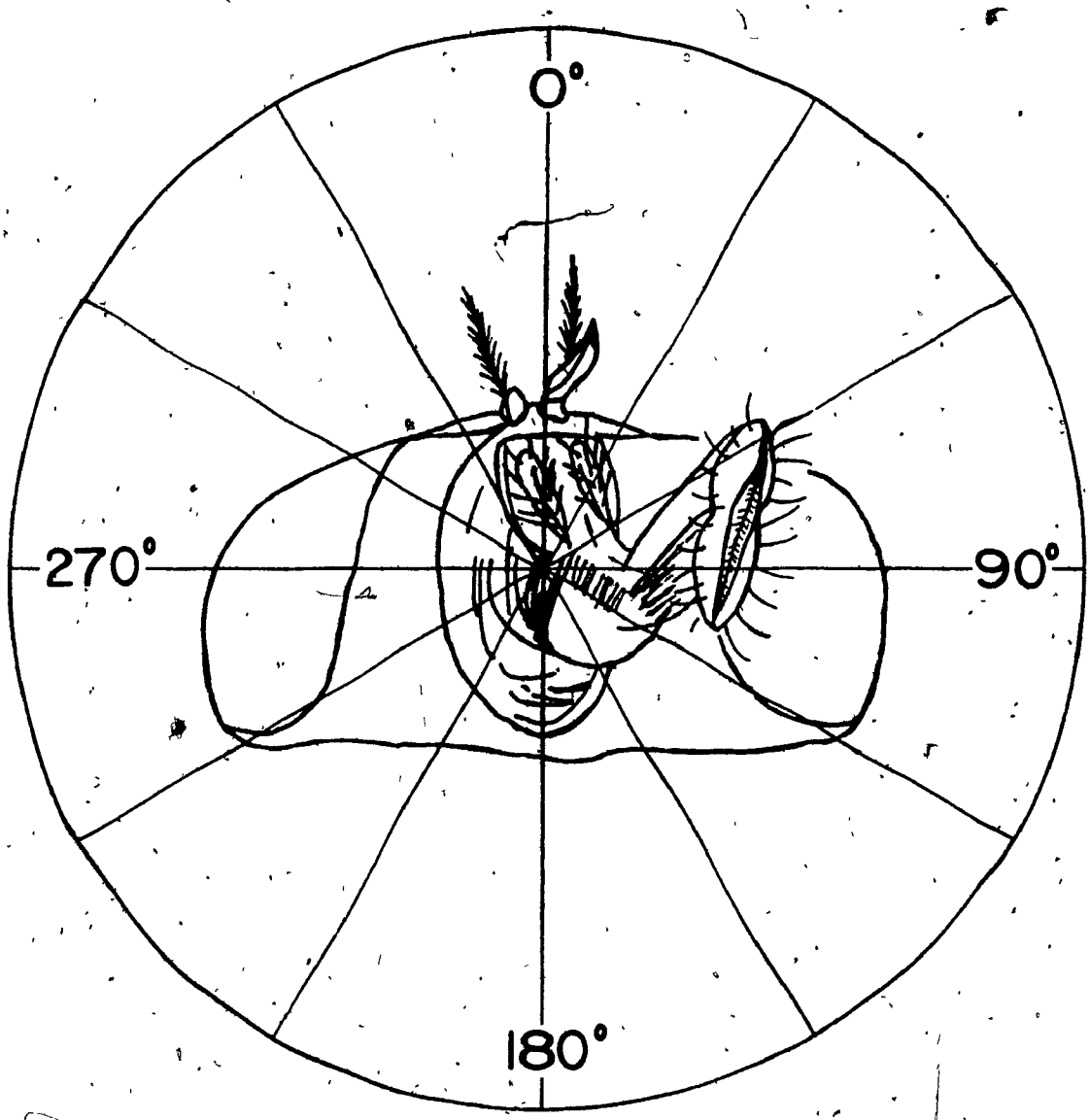

0°
270°
90°
180°

Fig. 2 Polar diagrams showing the distribution of the angles of extensions obtained with stimulation of hairs 2 (A), 6 (B) and 10 (C) with 1.0M sucrose. The lengths of the bars radiating from the circles are proportional to the number of responses at each direction. The arrows point to the mean directions of the responses, and the lengths of the arrows indicate the variability of the responses. N = sample size.

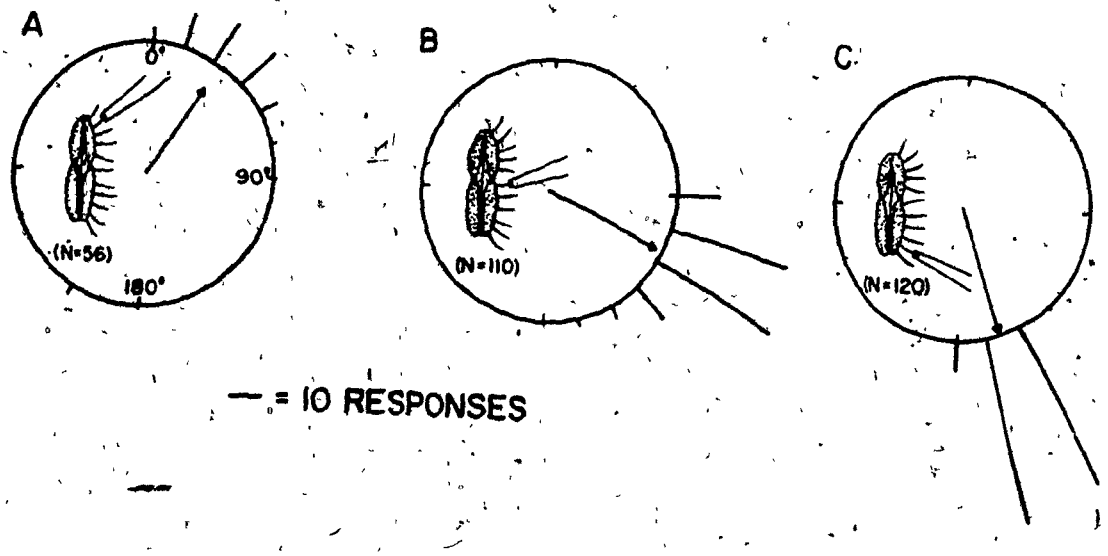

A
0°
90°
180°
(N=56)
B
(N=110)
C
(N=120)
— = 10 RESPONSES

Fig. 3 Summary diagram illustrating the mean response directions to stimulation of individual hairs with 1.0M sucrose. The hair corresponding to each arrow is indicated on the periphery of the circle. The sample sizes for each hair are: 53 (1), 56 (2), 68 (3), 96 (4), 154 (5), 110 (6), 141 (7), 130 (8), 129 (9), 120 (10), 41 (11).

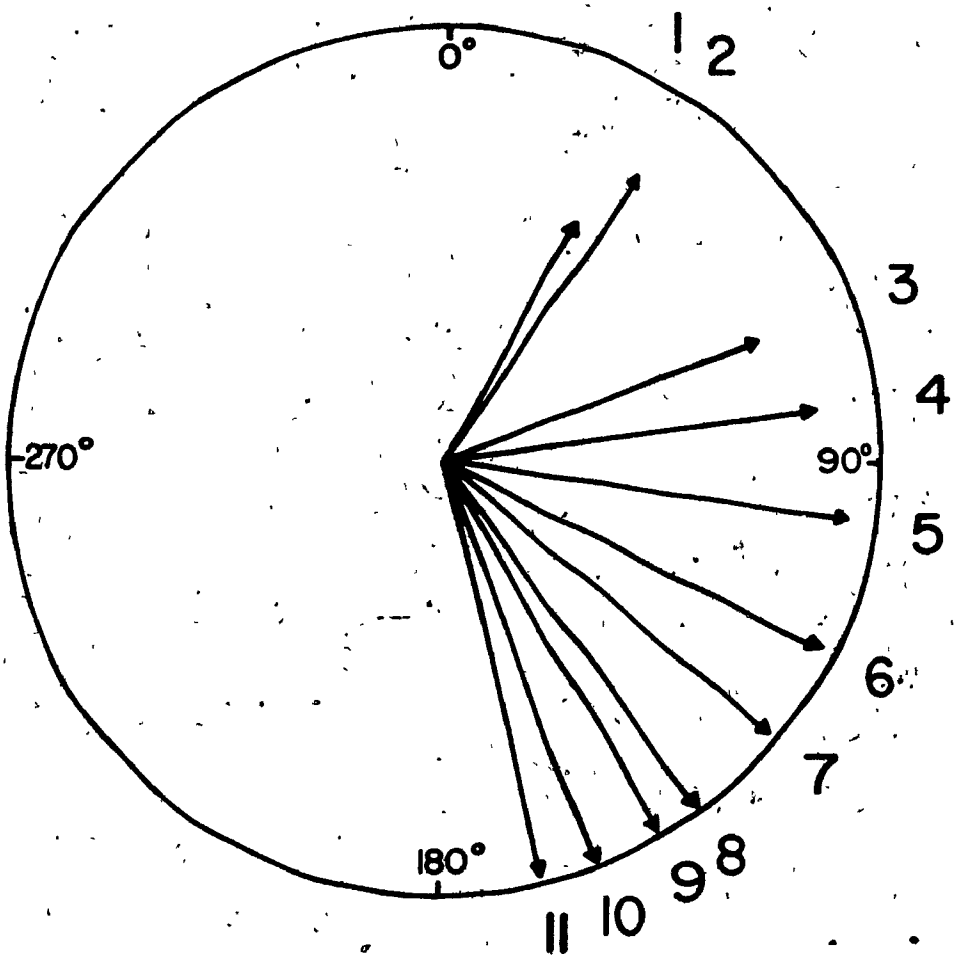

0°
90°
180°
270°
1
2
3
4
5
6
7
8
9
10
11

Fig. 4 Polar diagrams of the responses obtained with stimulation of two labellar hairs with 1.0M sucrose in CES-enhanced flies. For hairs 1+2, 2+3 and 3+4 the bimodal nature of the distributions of response directions is indicated. The hairs stimulated and the sample sizes are indicated within each diagram.

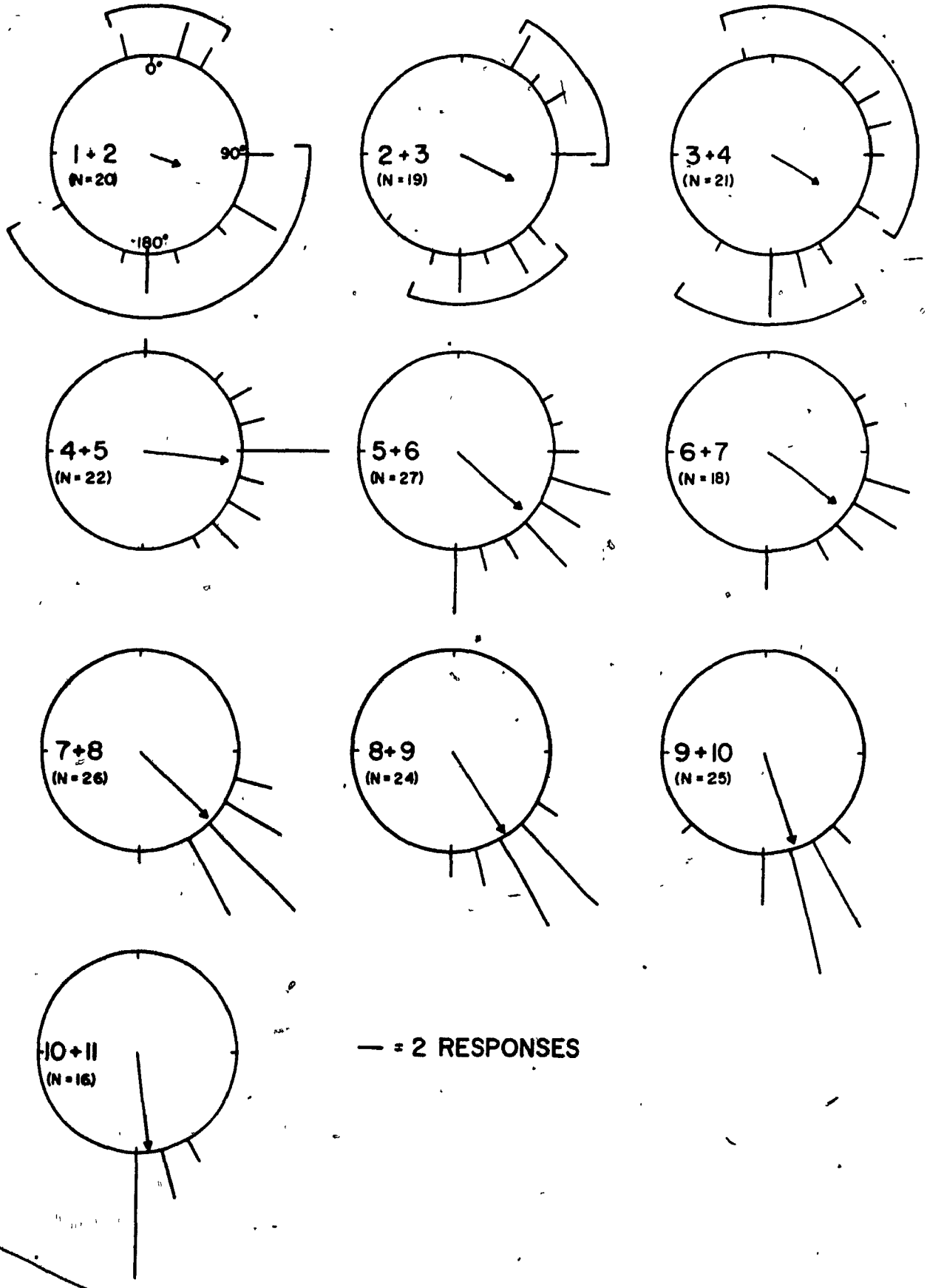

0°
90°
180°
1+2
(N=20)
2+3
(N=19)
3+4
(N=21)
4+5
(N=22)
5+6
(N=27)
6+7
(N=18)
7+8
(N=26)
8+9
(N=24)
9+10
(N=25)
10+11
(N=16)
— = 2 RESPONSES

Fig. 5 Polar diagrams of responses for the first five pairs of hairs, redrawn from Fig. 4. The responses are divided into two groups by the dashed line and the mean direction of each group is shown. The dashed arrows indicate the means of responses falling between 308° and 127° (the presumed hair-dependent group) and the solid arrows indicate the means of the responses between 128° and 307° (the presumed hair-independent group).

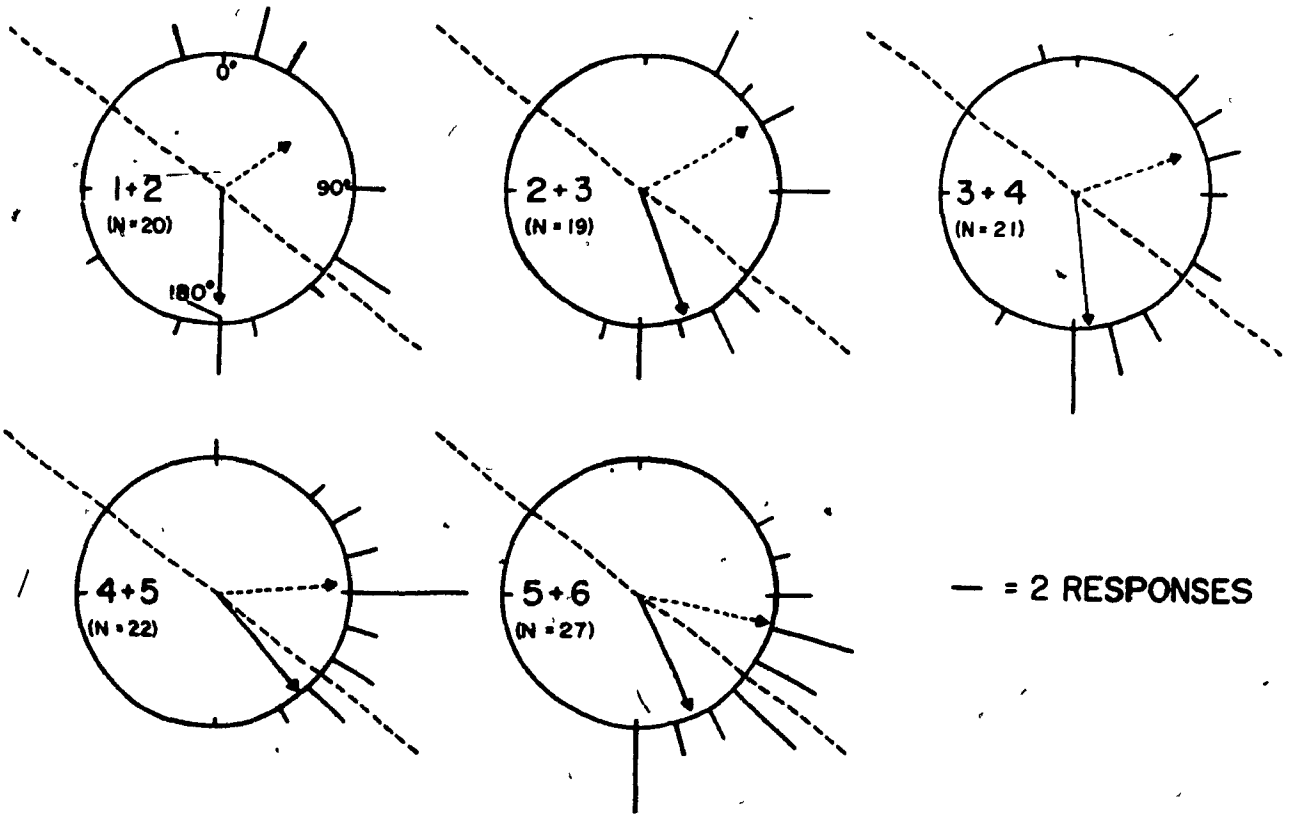

0°
90°
180°
1+2
(N=20)
2+3
(N=19)
3+4
(N=21)
4+5
(N=22)
5+6
(N=27)
— = 2 RESPONSES

Fig. 6 Summary diagram of the mean angles of extension obtained with simultaneous stimulation of two labellar hairs with 1.0M sucrose in CES-enhanced flies. The means illustrated for the first 5 pairs of anterior hairs (dashed arrows) were calculated from the responses falling between 308° and 127°; the means for the 5 posterior pairs of hairs (arrows with solid lines) were calculated from the whole population of responses. The hairs corresponding to each arrow are indicated on the periphery of the circle. The sample sizes are 13 (1+2), 9 (2+3), 10 (3+4), 18 (4+5), 13 (5+6), 18 (6+7), 26 (7+8), 24 (8+9), 25 (9+10), 16 (10+11).

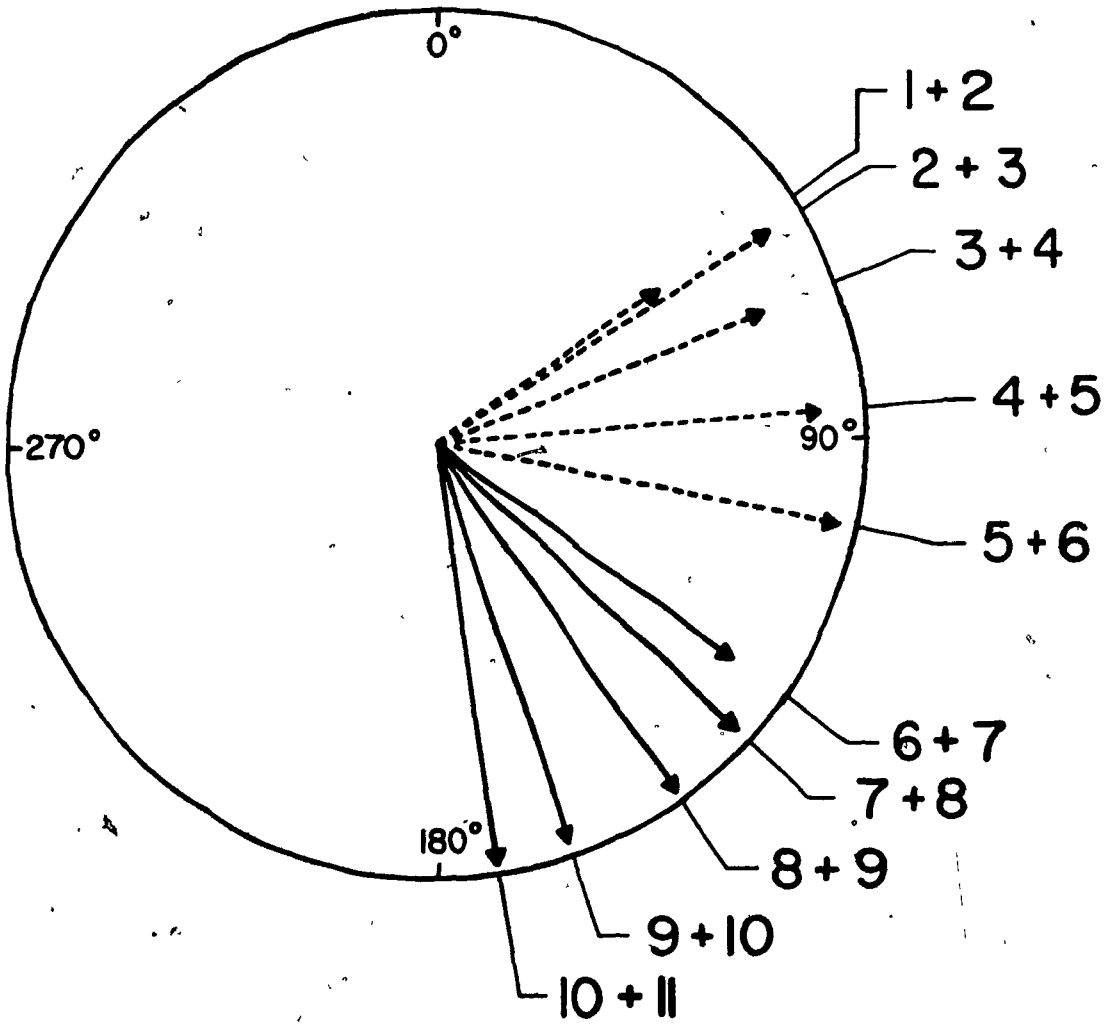
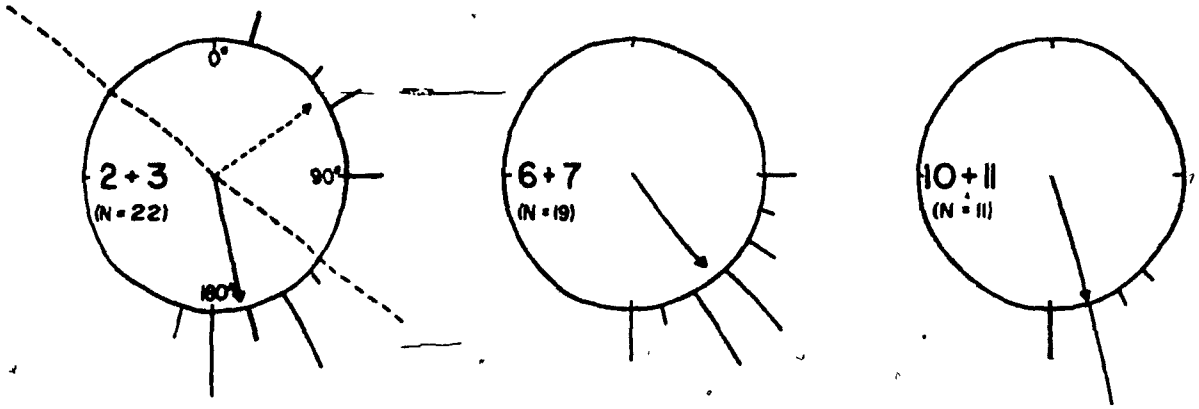
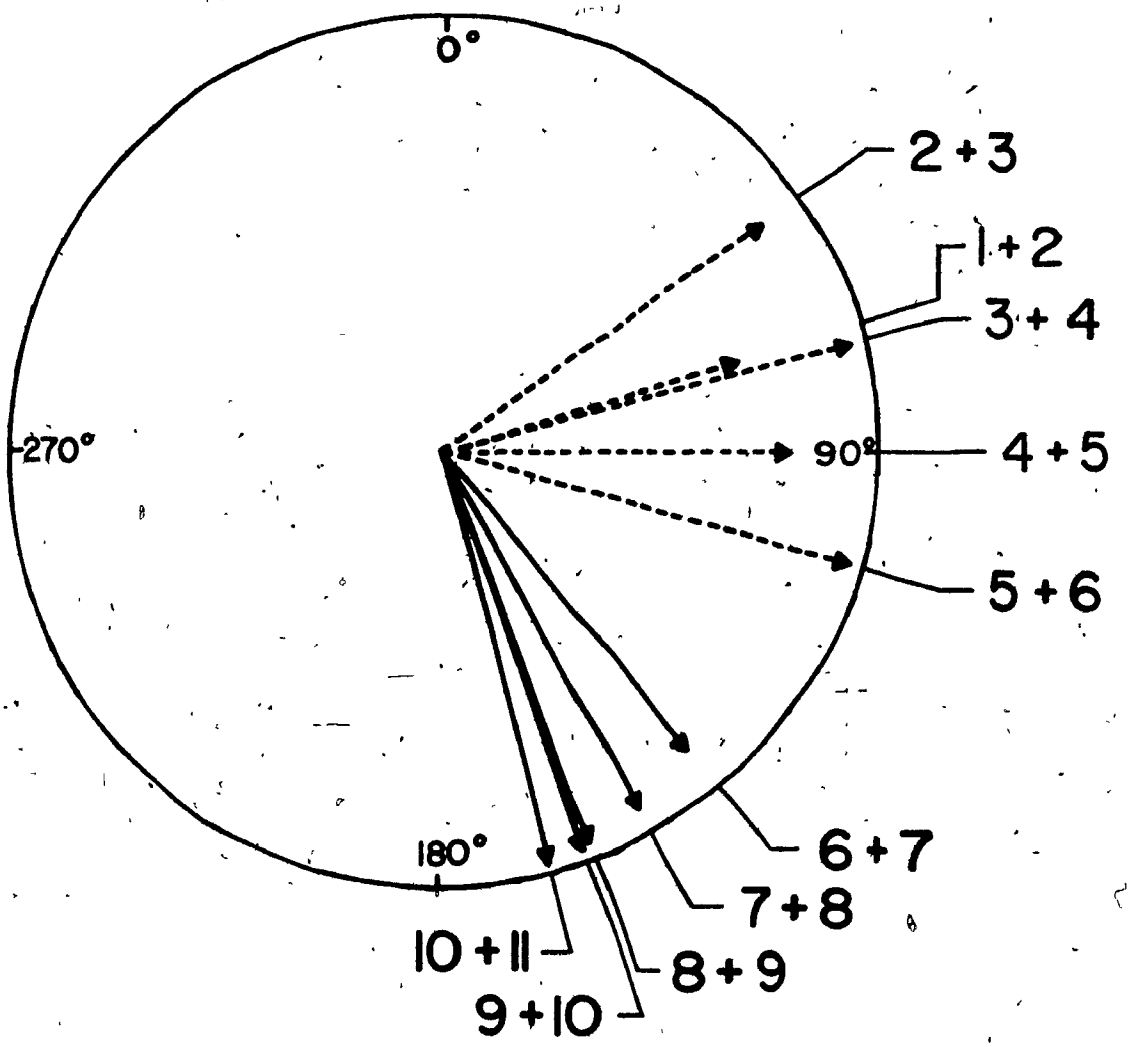

0°
90°
180°
270°
1+2
2+3
3+4
4+5
5+6
6+7
7+8
8+9
9+10
10+11

Fig. 7 Polar diagrams illustrating the responses obtained with simultaneous stimulation of two labellar hair with water in CES-enhanced flies, for hairs 2+3, 6+7 and 10+11. The responses to hairs 2+3 were divided into two groups as indicated by the dashed line, and the mean of each group was calculated separately; the arrow with the dashed line represents the mean of the responses falling clockwise of 308°, up to 127°, and the arrow with the solid line indicates the mean of the responses clockwise of 128°, up to 307°. For hairs 6+7 and 10+11, the arrows point to the means of the whole populations of responses.



— = 2 RESPONSES

Fig. 8 Summary diagram of the mean angles of extensions obtained with stimulation of two largest labellar hairs with water in CES-enhanced flies. The means for pairs 1+2 through 5+6 (arrows with dashed lines) were calculated for the responses falling between 308° and 127°, and the means for pairs 6+7 through 10+11 (arrows with straight lines) were calculated for all responses. The sample sizes are: 9 (1+2), 7 (2+3), 4 (3+4), 6 (4+5), 8 (5+6), 19 (6+7), 31 (7+8), 22 (8+9), 26 (9+10), 11 (10+11).


0°
270°
90°
180°
2+3
1+2
3+4
4+5
5+6
6+7
7+8
8+9
9+10
10+11

CHAPTER II

Central projections of labellar taste hairs in the blowfly, Phormia regina Meigen.

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ABSTRACT

We have traced the central projections of the receptor neurons associated with each of the eleven "largest" taste hairs on the labellum of the blowfly, Phormia regina (Meigen), by staining them with cobaltous lysine. The eleven hairs fall into three groups which reflect their peripheral locations and their branching patterns in the subesophageal ganglion. Group 1, consisting of the anterior hairs (numbers 1 and 2) and Group 3, consisting of the posterior hairs (numbers 9-11) project bilaterally, while Group 2, consisting of the middle hairs (numbers 3-8) projects primarily ipsilaterally. The central projections of the hairs within a single group are similar.

Each hair houses four chemoreceptors, which have differing chemical sensitivities and behavioral roles, and one mechanoreceptor. In some cases, there were indications that the different cells within a single hair have different central branching patterns. For some hairs, however, it was clear that a single central branching region and pattern was shared by more than one receptor cell.

We failed to find either a continuous somatotopic representation of a hair's position on the periphery, or an anatomical segregation of receptors coding for different

modalities. Behavioral experiments indicate that the fly is informed both of the identity of the hair stimulated and of the stimulus. Our results suggest that this information is not represented on a gross anatomical level.

KEY WORDS: Chemoreceptors - Central projections - Blowfly/-Somatotopic map - *Phormia regina*.

INTRODUCTION

Feeding behavior of the blowfly has long been a model system for studies of chemoreception. Several aspects of this behavior, including the physiology and ultrastructure of the chemoreceptors, the central integration of stimuli, the anatomy and physiology of the feeding apparatus, and the regulation of feeding by satiety cues have been thoroughly studied (see Dethier 1976 for review). A notable gap in our current understanding is the paucity of knowledge about the central neuroanatomy of chemoreceptors. The present paper provides an account of the projections in the central nervous system of one group of these chemoreceptors, those of the largest labellar hairs.

The fly possesses gustatory receptors on various regions of its body. Those that are most important for initiation of feeding are taste hairs that occur on the tarsi and mouthparts. Stimulating these hairs with an attractive stimulus causes the fly to extend its proboscis. Five neurons are associated with each taste hair (Peters and Richter 1965). Electrophysiological investigations have demonstrated that one of these is a mechanoreceptor and that the four remaining cells are contact chemoreceptors of different modalities. Because of their chemical sensitivities, these have been referred to as sugar, salt,

water and anion receptor cells (Dethier 1976). In Phoreia regina, there are over one hundred taste hairs on each of the bilaterally symmetrical labellar lobes. Among these are eleven 'largest hairs' (Wilczek 1967) that are arranged along an anteroposterior axis (Fig. 1). Because of their favorable position and size, and their identifiability from fly to fly, the largest hairs have been used extensively in electrophysiological and behavioral studies. Yetman and Pollack (1985) observed that stimulation of individual largest hairs results in a directed extension of the proboscis. The angle of extension within the horizontal plane depends on the identity of the hair stimulated, and corresponds to the position of that hair on the labellum's surface. Thus, the identities of the individual hairs are maintained within the central nervous system. The present study seeks to determine whether this individuality is reflected by the central anatomy of the neurons associated with the hairs. We show that the axons of the cells from the largest hairs branch and terminate in the subesophageal ganglion. Furthermore, we show that hairs that elicit differently directed proboscis extensions may share a common central branching pattern.

MATERIALS AND METHODS

Male and female blowflies, Phormia regina Meigen, of various ages were used. The central morphology of receptor cells contained in each of the eleven largest hairs was demonstrated by cobalt staining. The fly was immobilized, ventral side uppermost, by attaching its wings to a bed of wax with a warm needle. Its proboscis was gently withdrawn with forceps and stapled to a wax pillow. Wax was also used to hold the labellar lobes closed and immobile. A micropipet filled with 370mM cobaltous lysine (Springer and Prokosch 1982) containing 1% triton-X 100 was positioned so that the tip of the hair to be filled penetrated the solution. The stain thus had access to the dendrites of the receptor cells, which lie within the lumen of the hair, via its terminal pore (van der Wolk et al 1984). The stain remained in contact with the hair for 18 to 24 h at room temperature. The cobalt was then precipitated by soaking the dissected head ganglia for 10 min in physiological saline (Normann and Duve 1969) saturated with hydrogen sulfide. The tissue was fixed in Carnoy's solution, dehydrated in absolute alcohol, and cleared in methyl salycilate. Silver intensification was carried out according to Bacon and Altman's (1977) method. Whole-mount preparations were mounted in Canada Balsam. Selected preparations were embedded in soft Spurr's medium (Spurr

1969) and sectioned (10 or 14 μm in thickness) in the horizontal or sagittal planes. Some sections were counterstained with 0.1% methylene blue in 1% borax. Stained neurons were photographed and drawn with the aid of a drawing tube.

RESULTS

Fills of single hairs usually resulted in several (up to 4) cells being stained. It was usually impossible to make out the central branching pattern of each individual cell because the processes of different fibers overlapped extensively. Therefore, except where otherwise mentioned, the following descriptions refer to the overall central morphology revealed by all the fibers filled within one hair. The branching pattern formed from cells within any particular hair was found, in general, to be consistent from one preparation to another, although some variability, as described below, did occur. In the descriptions that follow, hairs are grouped according to their positions on the labellum and the similarity of their central projections.

Anterior hairs, 1 and 2

The following description is based on 12 fills of hair 1 and 22 fills of hair 2. A typical projection (from hair 1) is shown in Fig. 2A. Axons enter the subesophageal ganglion anteriorly and ventrally via the labial nerve. Just dorsal to the entry point they give off dense contralateral branches (labelled VB in Fig. 2A) and lateral branches in the ipsilateral hemiganglion (LB in Fig. 2A). More dorsally, a second, less dense group of fibers courses

medially (DB in Fig. 2A). Two fortuitous preparations in which only a single cell was stained suggest that different individual cells may contribute to different features of the overall pattern. The cell illustrated in Fig. 3A has both dorsal and ventral contralateral branches, but lacks a lateral projection. The cell in Fig. 3B has a ventral contralateral projection and a lateral branch, but no dorsal medial projection. In several multiple-cell fills, including the one shown in Fig. 2A, a cell with yet another morphology could be identified (Fig. 3C). The axon of this cell enters the subesophageal ganglion and travels dorsally, without branching, for approximately 150 μm. Then, at a level slightly dorsal to the previously described dorsal contralateral projection, it sends diffuse branches both laterally and medially, the latter crossing the midline. A cell with similar central anatomy was observed in at least one fill of each of the eleven largest hairs. Throughout this paper we will refer to cells of this type as Dorsalmost Bilaterally Projecting Cells (DBPC).

There was variability, both within and among hairs, in the details of the projection patterns. The ventral contralateral projection was observed in all 12 fills of hair 1, but in only 19 of the 22 fills of hair 2. In the remaining 3 cases the medially directed ventral fibers stopped at the midline. The dorsal contralateral branches (excluding those due to the DBPC) were observed in 9 of the

12 fills of hair 1 (in 1 case there was no indication of these branches; in 2 cases they were present, but failed to cross the midline) but in only 3 fills of hair 2 (in 15 cases the branches were present, but stopped at the midline, and in 4 cases this projection was extremely attenuated and consisted only of very short medially directed branches). The lateral branches were observed in 8 of the 12 fills of hair 1, and in all fills of hair 2. The DBPC was stained in 2 fills of hair 1, and in 6 fills of hair 2. Some of this variability (e.g., the presence or absence of a DBPC or of lateral branches) may be due to the capriciousness with which individual neurons are stained with cobalt. Other differences, however, such as the variable length of the dorsal and ventral contralateral projections, more likely reflect real variation in neuronal morphology. Despite this variability, the overall projection pattern is reasonably reliable. This is shown in Fig. 4A, in which drawings from 5 fills of hair 1 are superimposed.

The similarity of the projections from hairs 1 and 2 is demonstrated in Fig. 2B, which illustrates a preparation in which both the left hair 1 and the right hair 2 were filled. The possibility that the projections from these hairs might differ along the anteroposterior axis was tested by inspecting horizontal sections from ganglia in which hair 1 was filled on one side and hair 2 on the other. No difference was detected in the location of the two projections along the anteroposterior axis.

Mid-level hairs, 3-8

The following description is based on 12 fills of hair 3, 9 each of hairs 4, 5 and 6, 15 of hair 7 and 16 of hair 8.

Shortly after entering the subesophageal ganglion, in approximately the same region from which hairs 1 and 2 send their ventral contralateral fibers, the cells from hairs 3-8 give off a set of variably extensive posteriorly-directed fibers (Fig. 2D). The density of the posterior projection varied both within and among hairs, without apparent correlation with the identity of the hair. The main dorsally-coursing projection branches to form a 'V' or 'Y' according to the dorsoventral level of the branch point (Fig. 2C). Eight single-cell fills of various hairs all revealed similar 'Y' or 'V' patterns and posteriorly directed ventral branches, suggesting either that a single readily-filled cell type is responsible for this pattern, or that this branching pattern is common to several of the individual cells within each hair. The latter interpretation is supported by multiple-cell fills, in which it is sometimes possible to follow the major branches of more than one individual cell. In such cases, different cells from the same hair can clearly be seen to have similar branching patterns (Fig. 3D).

Several multiple-hair fills were performed in order to

gauge the similarities of the projections from hairs 3-8. Fig. 2E shows a ganglion in which hair 7 and hair 8 were filled on the left side and hair 4 was filled on the right side. The terminal arbors of cells from both hairs on the left side overlap extensively and project to the same region of their hemiganglion as the single hair filled on the right. The projections of different hairs within this group were not distinguishable, in horizontal sections, by their anteroposterior levels. Fig. 4B is a composite drawing of 5 fills of hair 6 which illustrates the consistency of the projection pattern.

Posterior hairs, 9-11

The following description is based on 21 fills of hair 9, 14 of hair 10 and 5 of hair 11. The central projections of these hairs are in many respects similar to those shown by the anterior hairs. A ventral contralateral projection was characteristically seen but, as for hairs 1 and 2, some variability was observed. The projection crossed the midline in all fills of hairs 10 and 11. For hair 9, the fibers crossed the midline in 18 cases, stopped at the midline in 2 cases, and stopped well within the ipsilateral hemiganglion in the remaining case. The projections of hairs 9 through 11 differed from those of the anterior hairs in that a dorsal contralateral projection was seldom observed. Only hair 10 showed this projection, and in only two preparations. In 7 cases the main dorsally-coursing branches sent sparse fibers which approached the midline but

remained ipsilateral. In the remaining fills of hair 10, and in all of the fills of hairs 9 and 11, the dorsal, medially directed projection was either extremely short or absent. Lateral branches, similar to those observed in the anterior hairs, were present in 7 fills of hair 9, in 2 fills of hair 10, and were never detected in fills of hair 11.

Fig. 2F is an example of a stain of hair 9 showing the characteristic features of this group and illustrating the DBPC as well. Two single-cell stains from posterior hairs, one from hair 10 (not shown) and one from hair 11 (Fig. 3E) had branching patterns similar to that observed in multiple-cell fills, including a ventral contralateral projection and a dorsally-coursing fiber that branched medially, crossing the midline in the cell from hair 10. Horizontal sections through ganglia in which different hairs were filled on either side reveal that the projections from hairs 9-11 lie at similar anteroposterior levels. The overall branching pattern of cells from this group of hairs is shown in Fig. 4C, in which 5 fills of hair 9 are superimposed.

DISCUSSION

Central representation of modality

Projections of labellar taste sensilla have been reported in Drosophila melanogaster. Stocker and Schorderet (1981) stained the receptor cells of labellar bristles with cobalt, and observed projections similar to those that we found for largest hairs 3-8 in Phormia regina. Nayak and Singh (1985), using a Golgi silver impregnation technique, described seven types of branching patterns for neurons associated with labellar sensilla. The mouthparts of D. melanogaster have three types of external chemosensilla (type-A bristles, type-B bristles, pegs) which contain respectively, four, two and one chemoreceptor each (Falk et al 1976). Nayak and Singh suggested that the seven morphological types that they observed correspond to the seven receptor cells found in these sensilla types. In the present paper we show that, in P. regina similar branching patterns can be expressed by more than one cell from a single sensillum (Fig. 3D). Thus, our results do not support the suggestion of Nayak and Singh. The five receptor cells contained within each of the largest hairs have been classified, based on electrophysiological studies, as sugar, salt, water, anion, and mechano- receptors (Dethier 1976). Our observations on the anterior hairs suggests that there may exist at least three morphologically

distinct cell types (Fig. 3A, B and C). In hairs 3 through 8, however, we found that more than one cell from the same hair can have similar projections (Fig. 3D). This suggests that cells which differ in their response specificity need not arborize in different regions of the ganglion. Because we have not been able to follow clearly the branches of more than two cells from any single-hair fill, it remains a possibility that more than two cells can have similar branching patterns. This is suggested by the fact that the central branching pattern obtained in multiple-cell fills did not differ substantially from the pattern formed by single cells for hairs 3-8. The possibility of a common termination region for several receptor types is especially intriguing because these receptors play different behavioral roles (Dethier 1976).

Central representation of peripheral position

A frequent finding for the organization of sensory systems is that the projections of sensory receptors in the central nervous system are spatially related to the positions of the receptors at the periphery. Such somatotopic maps have been found both in vertebrates (Jacobson 1978) and in invertebrates (Murphey et al. 1980; Ghysen 1980; Strausfeld and Nassel 1981; Romer 1983; Levine et al. 1985). The central projections of the labellar hairs show only a crude somatotopic organization. The anterior, mid-level, and posterior groups of hairs have different projection patterns, but within each of these groups

projections are similar.

The projection field of a sensory neuron is clearly important in determining the targets upon which it synapses. Bacon and Murphey (1984) showed that hairs on the cercus of the cricket, Acheta domesticus, which differ in their directional sensitivity to wind stimuli project to different regions of the terminal ganglion, where they are sampled by different postsynaptic neurons. We have described three groups of central patterns formed by the 11 hairs on the labellum. Projections from the anterior hairs, 1 and 2, and the posterior hairs, 9-11, are strongly bilateral, whereas in the mid-level hairs, 3-8, the projections are mainly restricted to the ipsilateral hemiganglion (excepting the DBPC). It seems possible that these differences among projection patterns may be related to differences in the postsynaptic targets of the cells. Although we have not yet identified these targets, our behavioral observations (Yetman and Pollack 1985) are consistent with this notion. Stimulation of the anterior and posterior hairs results in proboscis extensions which are directed close to the midline. We presume that such extensions are accomplished by relatively symmetrical activation of muscles on the left and right sides. Bilaterally symmetric muscle activity would correlate well with the strong bilateral projections of the neurons from these hairs. Similarly, stimulation of the middle hairs, the projections of which are primarily

ipsilateral, results in laterally-directed extensions, which presumably require more asymmetric muscle activity. A similar correlation between the occurrence of unilateral or bilateral projections and the degree of symmetry or asymmetry of muscle activation has recently been reported for receptors which elicit abdominal flexion reflexes in caterpillars (Levine et al. 1985).

The termination regions of the chemosensory neurons cannot, however, fully account for the directions of proboscis extension elicited by stimulation of individual hairs. Hairs which have similar central projections (e.g., hairs 3 and 8) can result in differently directed extensions of the proboscis (Yetman and Pollack 1985). Thus, while the anatomical projections of the hairs form a discontinuous somatotopic map of the periphery (they change abruptly between hairs 2 and 3, and again between hairs 8 and 9) the proboscis extensions elicited by stimulating these hairs are continuously graded in direction. This might be explained if cells that terminate in the same region (e.g. those from hairs 3 and 8) form synaptic connections of differing strengths onto a common pool of follower cells. Such an organization would be similar to that which has been described for the cercal system of the cockroach, where the directional sensitivity of wind-sensitive giant interneurons is thought to be due to the differing strengths of synapses made by receptor cells stimulated by winds from different directions (Daley 1982).

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Fig. 1. Diagram of right labellar lobe (after Wilczek 1967) illustrating the positions of the largest hairs. Number one is anterior and number eleven is posterior.

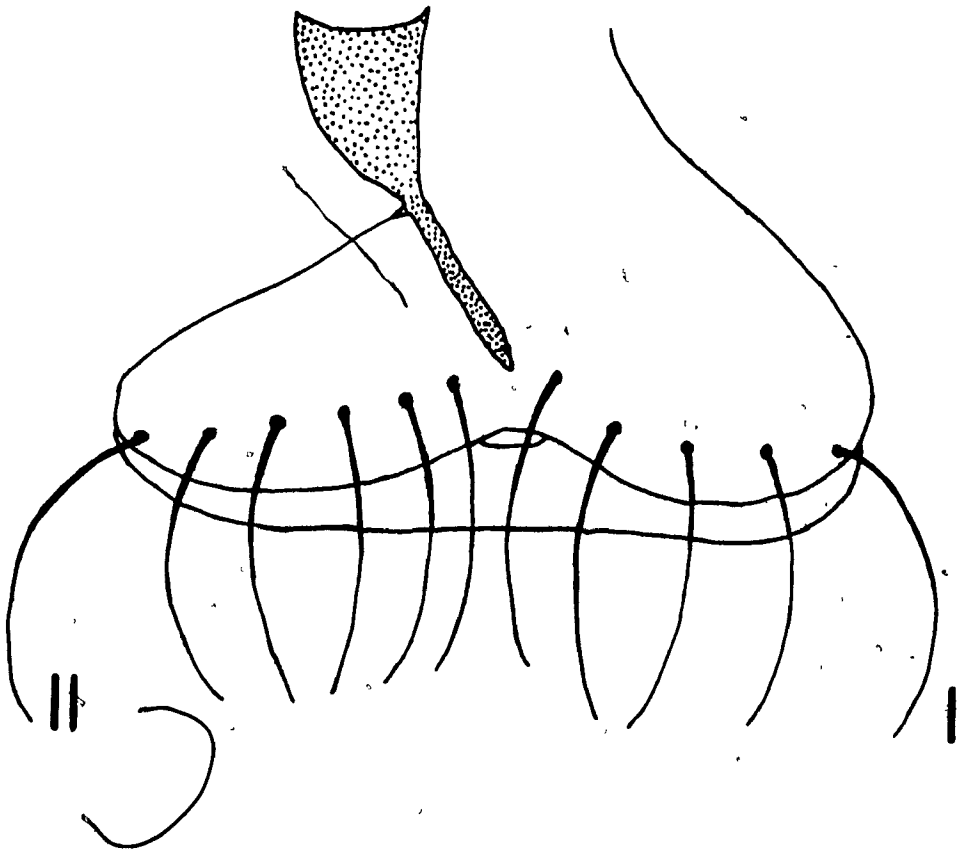

Fig. 2. Photographs of cobalt-filled sensory cells. A,B,C,E and F are frontal views of wholemounts; dotted lines indicate midline. D is a sagittal section. x300

A Branching pattern of cells contained in hair 1. VB ventral contralateral branches, DB dorsal contralateral branches, LB lateral branches, DBPC dorsal-most bilaterally projecting cell.

B The similarities between the central patterns formed by stains of hairs 1 and 2 is demonstrated in this double fill.

C Central projections from hair 3; montage of photographs taken at two different planes of focus. PB posterior branches.

D Sagittal section (14 um) through the subesophageal ganglion of an animal in which hair 8 was filled. Arrow points to posteriorly projecting ventral branches. A anterior, P posterior, D dorsal V ventral.

E Preparation in which hair 4 was filled on the right side and hairs 7 and 8 were filled on the left side.

F Projection from hair 9. DBPC dorsal-most bilaterally projecting cell.

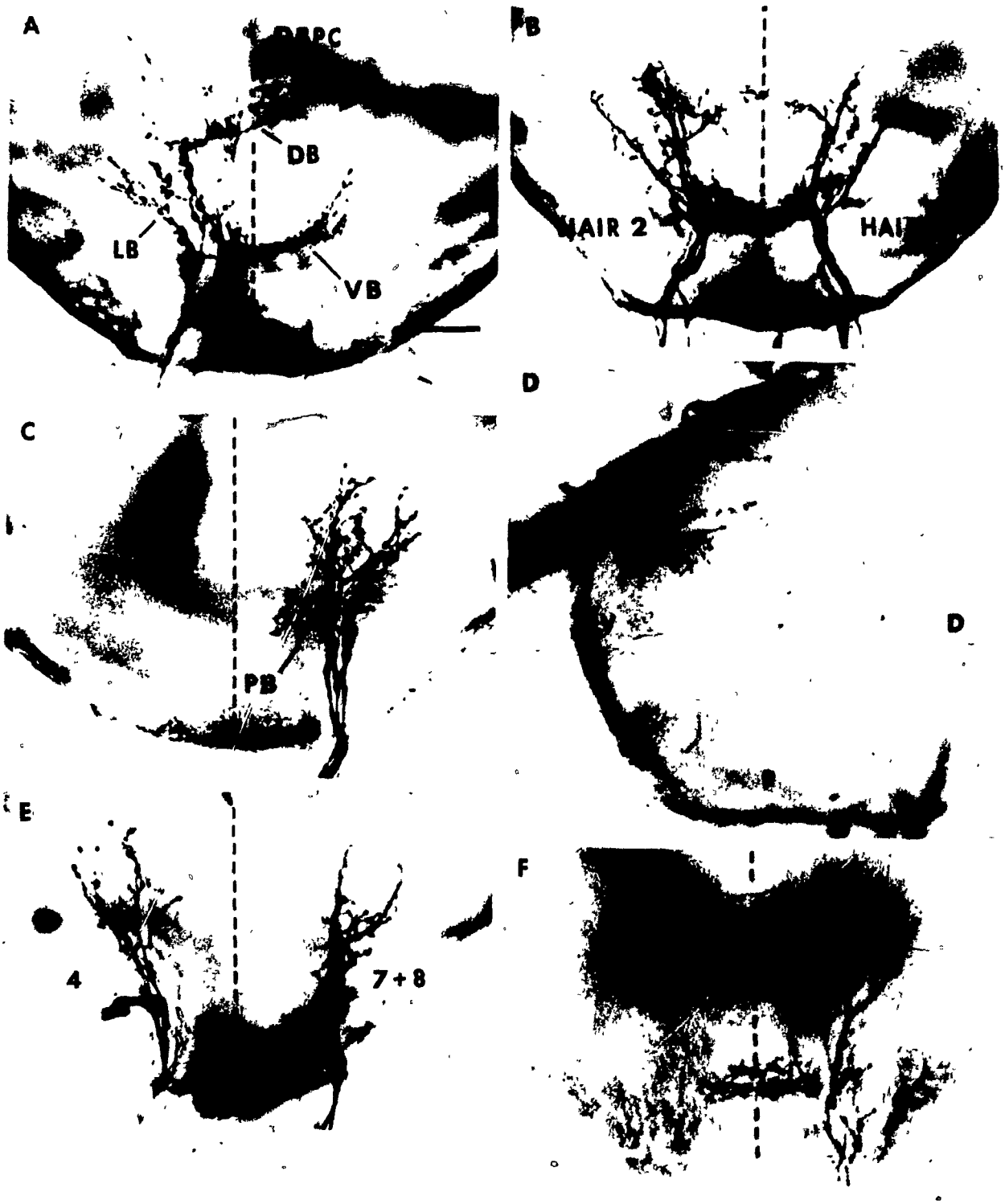
A
DBPC
DB
LB
VB
B
HAIR 2
HAI
C
PB
D
D
E
4
7+8
F

Fig. 3 Drawings of single cells, frontal view. x300

A and B single-cell fills of hair 1.

C DBPC drawn from a fill of hair 1.

D Branching pattern formed by two individual cells from a fill of hair 4 are resolved in this drawing. To illustrate best the different branches of the two cells, one was drawn with a thick pen and the other with a thin pen.

E Drawing of a fill of hair 11 in which a single cell was stained.

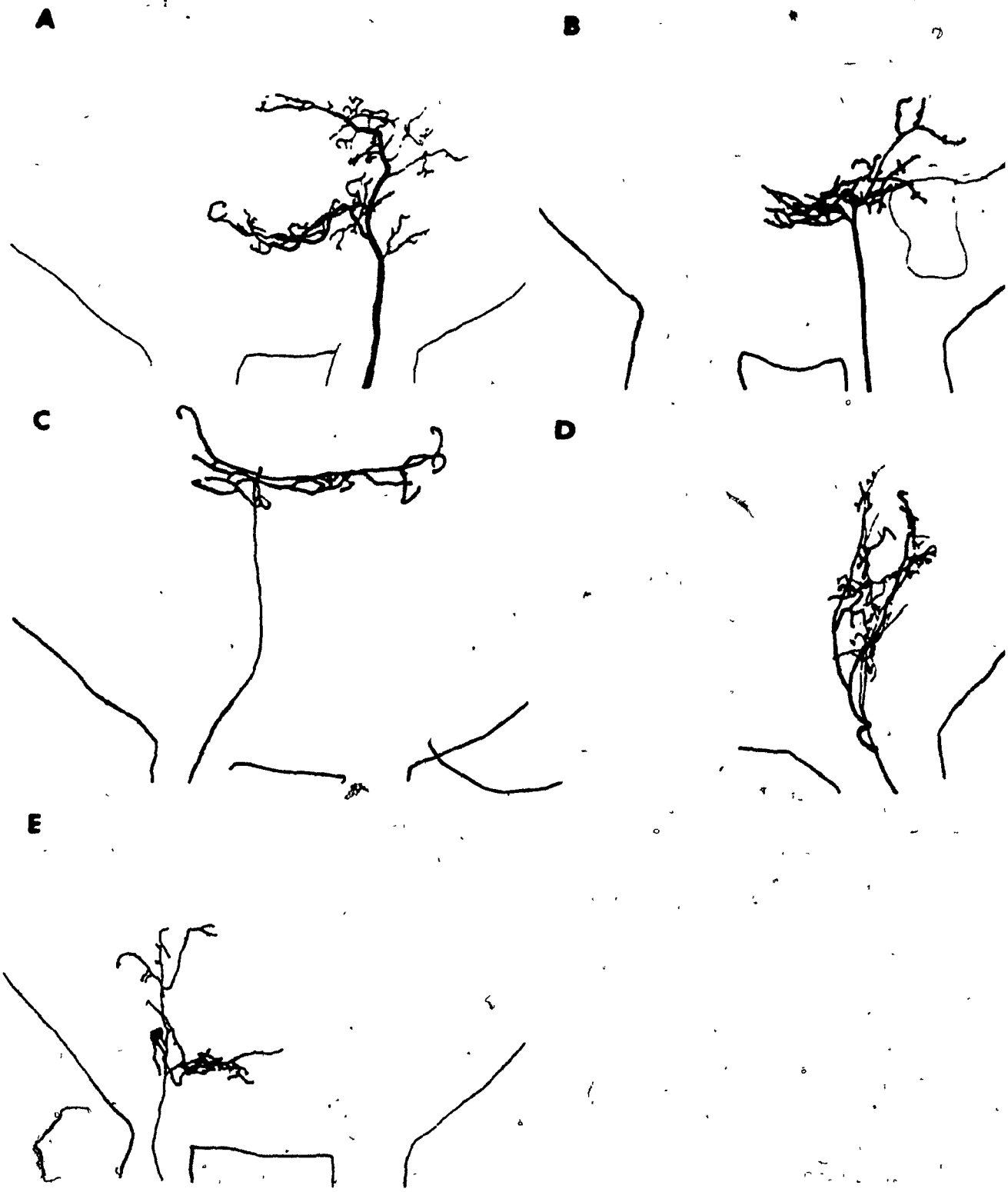
A
B
C
D
E

Fig. 4 A Superposition of 5 drawings of fills of hair 1.

B Superposition of 5 drawings of fills of hair 6.

C Superposition of 5 drawings of fills of hair 9. x300


A
B
C

CONCLUDING REMARKS

Organisms perform a variety of behaviors which increase their efficiency of sampling the chemicals in their environment, enhancing their chances of locating stimuli released by food or conspecifics. For examples, the cockroach waves its antennae when it detects a source of pheromone (Rust, 1976), the Surphid larvae swings its anterior segments sideways and forward and back after it contacts an aphid (Chandler, 1969). These active responses to chemical stimulation increase the sampling rate and sampling field of the chemicals. To follow a trail, the snake determines the areas of highest concentration by sampling the substrate with its tongue. The tongue is extended out and from side to side collecting the necessary information to maintain the snake along the trail (Schöne, 1984). A small drop of sugar stimulates the onset of a similar behavior in a hungry fly. The proboscis is extended and retracted reflexively while the fly goes over the area where it had first found the stimulating drop (Dethier, 1957; Nelson, 1977; White et al., 1984). While the proboscis is extended, gustatory receptors on its terminal segment, the labellum, sample the walking substrate for food. I have shown that when some labellar hairs are stimulated, information concerning the location of the food source is encoded in the cells innervating them.

Furthermore, this information can be used to steer the proboscis towards the stimulated direction. During the dance, when some hairs contact a food patch, information about the identities of the hairs stimulated can be used to orient the proboscis over the patch and perhaps steer the body towards the stimulated side.

Often where external receptors have a recognizable spatial innervation pattern, investigators have looked for a central representation of their ordered peripheral topography. The vertebrate somatosensory system for example is spatially represented in the somatosensory cortex (Werner and Whitsel, 1973). Similarly, mechanoreceptors on the cercus of the cricket possess synaptic fields which are dependent on their peripheral position (Murphey et al. 1980). A point-to-point representation of the retina onto the superior colliculus has been demonstrated in the frog (Gaze, 1958), pigeon (Hamdi and Whitteridge, 1954), cat (Apter, 1945) and other vertebrates. Recently, Morita and Finger (1985) have shown that the gustatory receptors in the goldfish's mouth also maintain a spatial topography centrally. In this study I have shown that although the gustatory largest hairs of the fly are spatially arrayed peripherally, the synaptic field of the cells innervating them do not show a similar spatial continuum, instead, three discontinuous projection fields have been demonstrated. The behavioral experiments however have demonstrated that the hairs are indeed recognized individually centrally; each

hair eliciting a different direction of proboscis extension. Together, the results suggest that the terminal field of sensory neurons is not the sole determinant of their connectivity.

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