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

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Summary. 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 chemical nature of the stimulus. Our results suggest that this information is not represented on a gross anatomical level.

Key words: Chemoreceptors – Central projections – Somatotopic map – *Phormia regina* (Blowfly)

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 throughly 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.

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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 *Phormia 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 micropipette filled with 370 mM 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

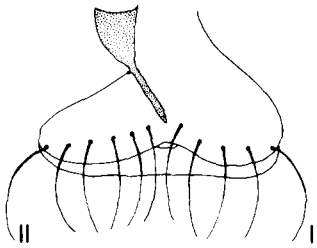

Fig. 1. Diagram of right labellelar lobe (after Wilczek 1967) illustrating the positions of the largest hairs. Number one is anterior and number eleven is posterior

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 (Norman and Duve 1969) saturated with hydrogen sulfide. The tissue was fixed in Carnoy's solution, dehydrated in absolute alcohol, and cleared in methyl salicylate. 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 um. 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 'Dorsal-most 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 gangion, 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

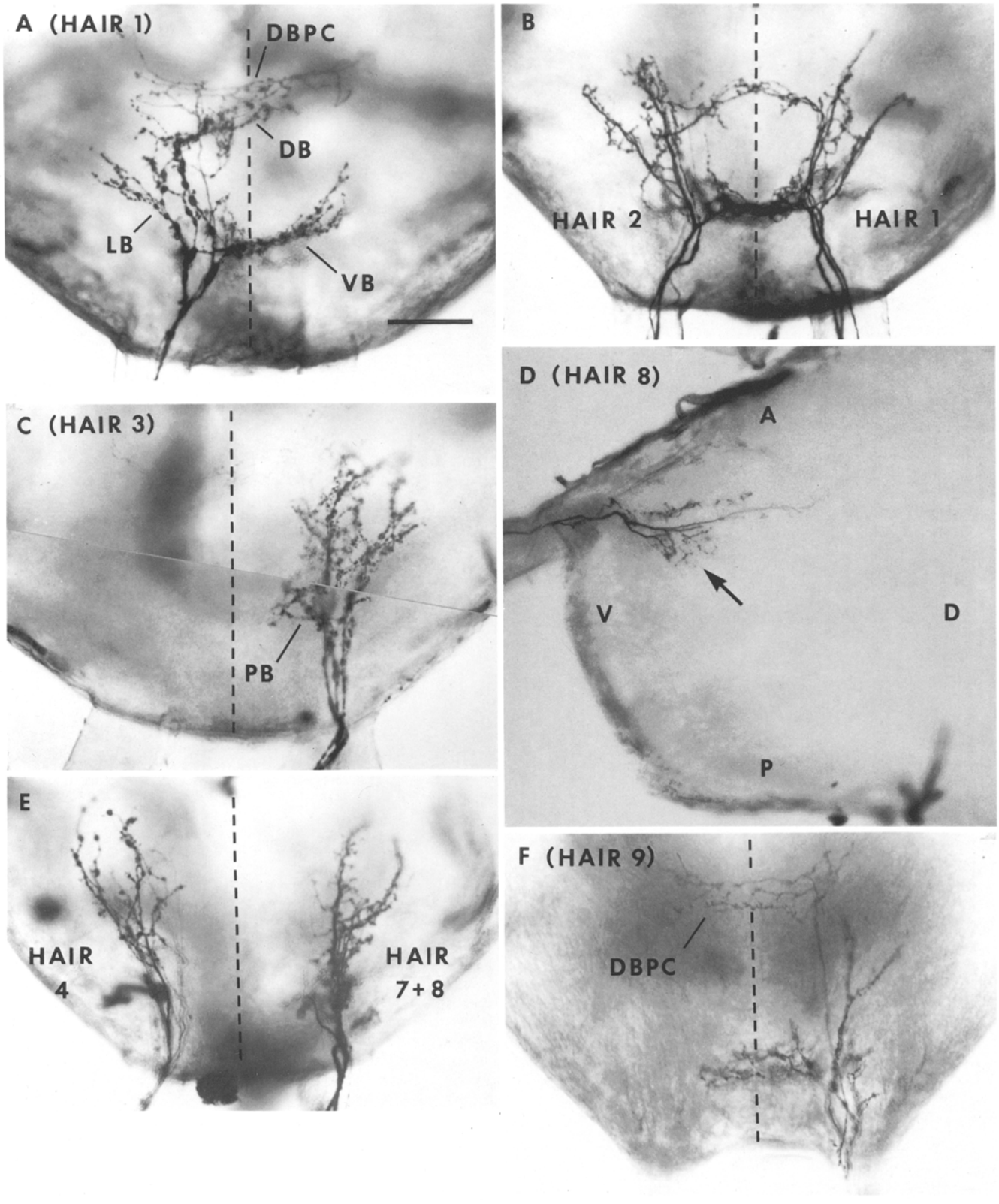

Fig. 2. Photographs of cobalt-filled sensory cells. **A, B, C, E** and **F** are frontal views of wholemounts; *dotted lines* indicate midline. **D** is a sagittal section. × 300 **A** Branching pattern of cells contained in hair 1. *VB* ventral contralateral branches, *DB* dorsal contralateral branches, *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 μm) 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

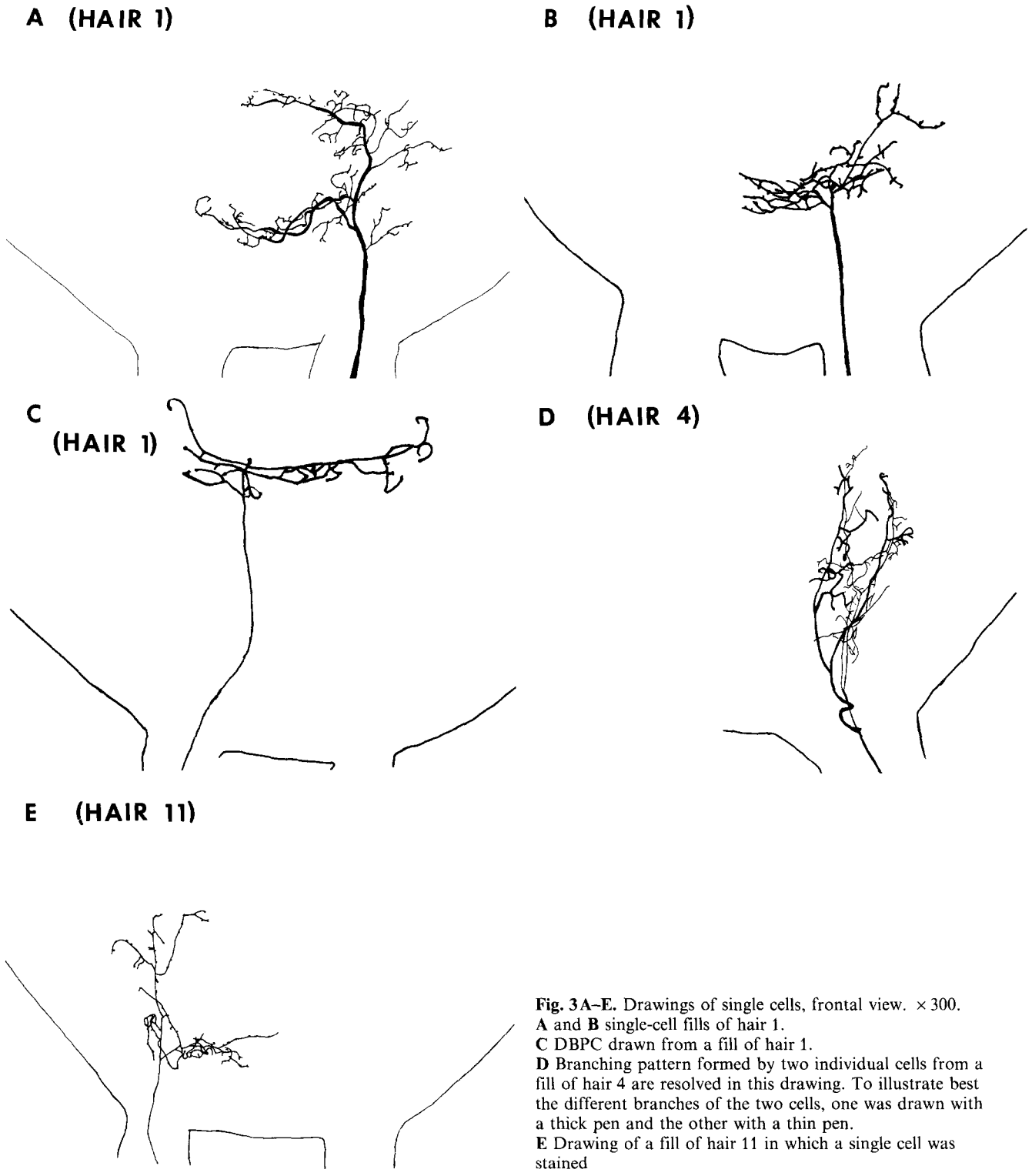

Fig. 3A–E. Drawings of single cells, frontal view. × 300.
A and **B** single-cell fills of hair 1.
C DBPC drawn from a fill of hair 1.
D Branching pattern formed by two individual cells from a fill of hair 4 are resolved in this drawing. To illustrate best the different branches of the two cells, one 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

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 antero-posterior levels. Fig. 4B is a composite drawing of 5 fills of hair 6 which illustrates the consistency of the projection pattern.

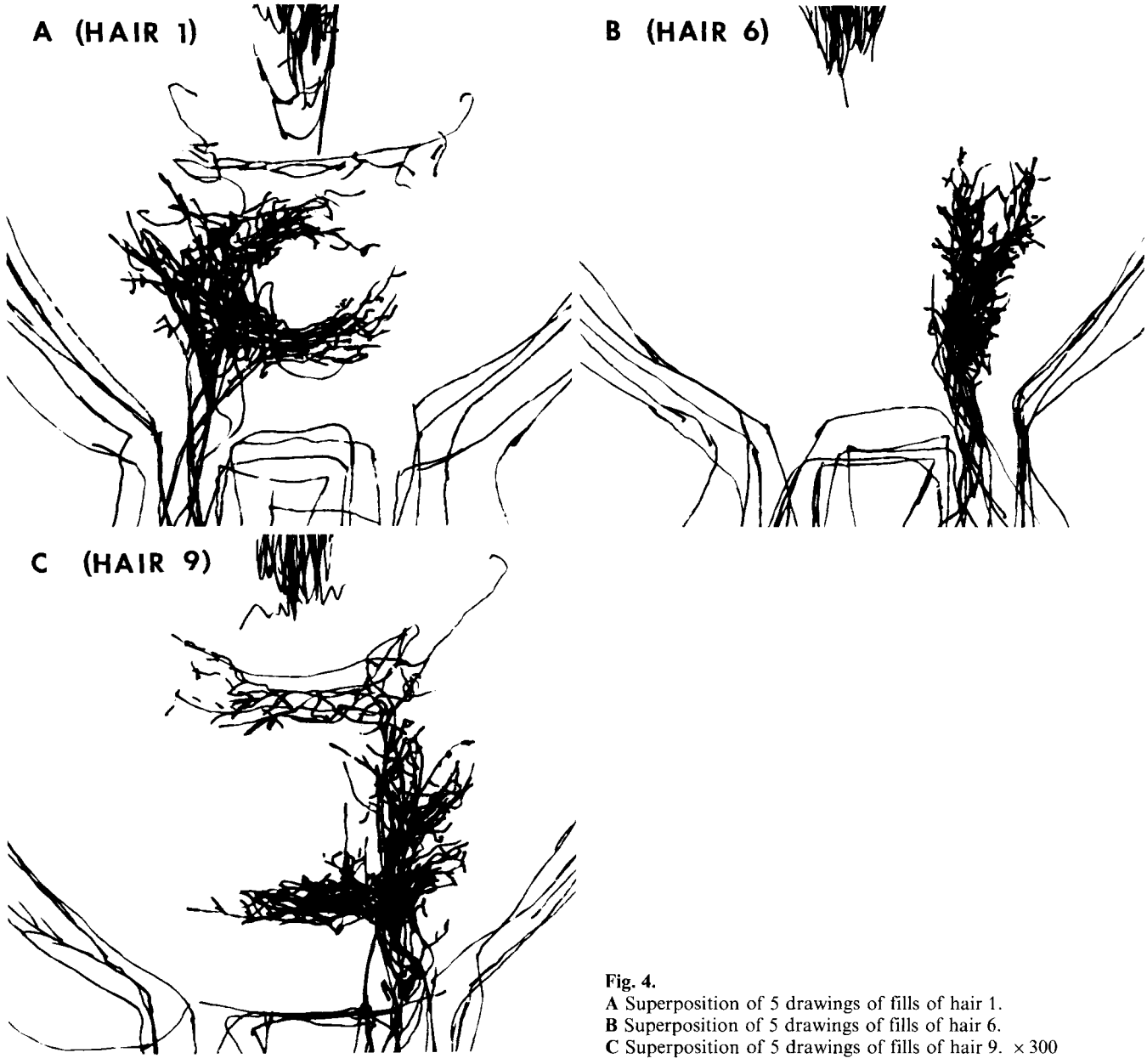

Fig. 4.
A Superposition of 5 drawings of fills of hair 1.
B Superposition of 5 drawings of fills of hair 6.
C Superposition of 5 drawings of fills of hair 9. × 300

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 contains 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 suggest that there may exist at least three morphologically distinct cell types (Fig. 3A–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 Nässel 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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References

Bacon JP, Altman JS (1977) A silver intensification method for cobalt-filled neurones in wholemount preparations. Brain Res 138:359–363

Bacon JP, Murphey RK (1984) Receptive fields of cricket giant interneurones are related to their dendritic structure. J Physiol 352:601–623

Daley DL (1982) Neural basis of wind-receptive fields of cockroach giant interneurons. Brain Res 238:211–216

Dethier VG (1976) The hungry fly. Harvard University Press' Cambridge, Massachusetts

Falk R, Bleiser-Avivi N, Atidia J (1976) Labellar taste organs of *Drosophila melanogaster*. J Morphol 150:327–342

Ghysen A (1980) The projection of sensory neurons in the central nervous system of *Drosophila*: Choice of the appropriate pathway. Develop Biol 78:521–541

Jacobson M (1978) Developmental neurobiology. Plenum Press, New York, pp 345–433

Levine RB, Pak C, Linn D (1985) The structure and metamorphic reorganization of somatotopically projecting sensory neurons in *Manduca sexta* larvae. J Comp Physiol 157:1–13

Murphey RK, Jacklet A, Schuster L (1980) A topographic map of sensory cell terminal arborizations in the cricket CNS: Correlation with birthday and position in a sensory array. J Comp Neurol 191:53–64

Nayak SV, Singh RN (1985) Primary sensory projections from the labella to the brain of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Int J Insect Morphol Embryol 14:115–129

Normann TC, Duve H (1969) Experimentally induced release of a neurohormone influencing hemolymph trehalose level in *Calliphora erythrocephala* (Diptera). Gen Comp Endocrinol 12:449–459

Peters W, Richter S (1965) Morphological investigations on the sense organs of the labella of the blowfly, *Calliphora erythrocephala* Mg. Proc XVI Int Congr Zool Washington 3:89–92

Romer H (1983) Tonotopic organization of the auditory neuropile in the bushcricket *Tettigonia viridissima*. Nature 306:60–62

Springer AD, Prokosch JH (1982) Surgical and intensification procedures for defining visual pathways with cobaltous-lysine. J Histochem Cytochem 30:1235–1242

Spurr AR (1969) A low-viscosity epoxy embedding medium for electron microscopy. J Ultrastruct Res 26:31–43

Stocker RF, Schorderet M (1981) Cobalt filling of sensory projections from internal and external mouthparts in *Drosophila*. Cell Tissue Res 216:513–523

Strausfeld NJ, Nässel DR (1981) Neuroarchitectures serving compound eyes of Crustacea and insects. In: Autrum H (ed) Vision in invertebrates. Springer, Berlin Heidelberg New York (Handbook of sensory physiology, vol VII/6b) pp 1–132

Wilczek M (1967) The distribution and neuroanatomy of the labellar sense organs of the blowfly *Phormia regina* Meigen. J Morphol 122:175–201

Wolk FM van der, Koerten HK, van der Starre H (1984) The external morphology of contact-chemoreceptive hairs of flies and the motility of the tips of these hairs. J Morphol 180:37–54

Yetman S, Pollack GS (1985) Proboscis extension in the blowfly: positional effects of chemoreceptors. Soc Neurosci Abst 11:1219

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