Birth Times of Neurons in Labellar Taste Sensilla of the Blowfly *Phormia regina*

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SUMMARY

We studied the birth times of neurons of labellar taste sensilla in blowflies using incorporation of the thymidine analogue 5-bromodeoxyuridine (BrdU) as an indicator of birth time. We found that one of the two main sensillum types, the taste papillae, arise according to a clear spatial gradient of birth times, whereas the other sensillum type, taste hairs, arise without any apparent spatial ordering. Within each sensillum type, there was a strong tendency for either all or none of the neurons to have incorporated BrdU. Among those rare sensilla in which only some of the neurons incorporated BrdU, there were clear patterns of the distribution of labeled and unlabeled neurons per sensillum. These results suggest that subsets of the neurons of a sensillum are siblings, and thus argue against the possibility that the several neurons of a sensillum arise from a single stem cell precursor through repeated asymmetrical divisions.

Keywords: chemoreceptor, fly, lineage, 5-bromodeoxyuridine, metamorphosis.

INTRODUCTION

Flies possess two types of external taste sensilla: taste hairs, which are found on the aboral margins of the labellar lobes as well as on the legs, wings, and ovipositor, and taste papillae, which occur on the oral surface (Dethier, 1976). Each of these sensillum types is multiply innervated, and the different component neurons are characteristically responsive to different stimuli. For example, labellar and tarsal taste hairs of *Phormia regina* generally contain five neurons, which can roughly be classified as a mechanoreceptor, a sugar receptor, a water receptor, a "classic" salt receptor, and a poorly understood fifth cell, which is sometimes classified as an anion receptor (Dethier, 1976). The taste papillae usually house either three or four neurons, of which one is a mechanoreceptor, one a sugar receptor, one a classic salt receptor, and the fourth is unclassified (Dethier and Hanson, 1965).

The different stimulus sensitivities of taste receptors are associated with differences in projection sites in the central nervous system (Yetman and Pollack, 1986; Possidente et al., 1989; Edgecomb and Murdock, 1992; Nottebohm et al., 1992) and with differences in the behavioral responses they elicit (Dethier, 1976). Thus, the neurons of a sensillum exhibit distinct phenotypes.

External sensilla of insects (which include, in addition to one or more neurons, several nonneuronal accessory cells) usually (but not always; see Technau and Campos-Ortega, 1986; Hartenstein and Posakony, 1989) comprise a clone derived from a single epithelial cell, the sensory mother cell (see Bate, 1978, for review). Earlier work has shown that the two progeny of the first division of the sensory mother cell of taste sensilla are, respectively, a precursor of two of the accessory cells (the trichogen and tormogen cells) and a precursor that gives rise to a third accessory cell (the thecogen cell) as well as to the neurons (Peters, 1963; Hartenstein and Posakony, 1989; Ray et al., 1993). The sequence of divisions by which this second
precursor generates the group of five neurons has been studied recently for taste hairs of *Drosophila* (Ray et al., 1993). Nothing is yet known of the sequence of divisions that generates the neurons of taste papillae.

Two extreme lineage types are possible that could generate a group of neurons from a single precursor. First, the precursor might act as a stem cell, undergoing repeated asymmetrical divisions with each round of mitosis producing a cell that would differentiate into one of the neurons (or into the thecogen cell) and a next-generation precursor. Iteration of this scheme would produce a series of differentiated cells of differing ages. Alternatively, the precursor might divide to produce daughters that would themselves be precursors. These might undergo one or more additional rounds of symmetrical divisions (symmetrical in the sense that both daughters are either next-stage precursors or cells that will differentiate into one of the components of the sensillum), with intermediate generations producing additional pairs of precursors and the final division producing daughters that would differentiate into either neurons or the thecogen cell. These different lineage types could be associated with different modes of determination of cellular phenotypes. For example, if the stem cell type of lineage occurred, then the differentiated phenotype of each cell might be specified by its relative time of birth with, e.g., the oldest cell determined to differentiate into the mechanoreceptor, the next oldest into the sugar receptor, and so on. Determination of phenotype by position within a stem cell lineage has been demonstrated for some central neurons of insects (Taghert et al., 1984; Doe and Goodman, 1985). If, however, differentiated cells were similar in age, as would be the case if they were produced by symmetrical mitoses, then rela-
Birth Times of Taste Sensilla

Table 1 Number of Neurons per Taste Hair

<table>
<thead>
<tr>
<th>Injection Time</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>3.8</td>
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<td>81.0</td>
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<td>89.2</td>
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</table>

Control is based on data from three preparations, others
are based on two preparations each. For each preparation, all
hair sensilla which were morphologically accessible, i.e., not ob-
scured by other tissue, were scored. Only preparations that repre-
sented all regions of the labellum were used.

Table 2 Number of Neurons per Lateral Papilla

<table>
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<tr>
<th>Injection Time</th>
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Table 3 Number of Neurons per Interpseudotracheal Papilla

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<th>4</th>
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<td>78</td>
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<tr>
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<td>4.4</td>
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<td>113</td>
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<td>12.7</td>
<td>83.5</td>
<td>1.3</td>
<td>79</td>
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</tbody>
</table>

Number of preparations per treatment as for Table 2.

MATERIALS AND METHODS

P. regina (Meigen) were reared at 25°C under a
12:12 h light/dark cycle. Adults were fed sucrose, water,
and beef liver, and larvae were reared on an artificial
medium consisting of milk, yeast, and agar (modified
from Hill et al., 1947). Under these conditions, first in-
star larvae hatch about 20 h after egg laying, pupariation
occurs about 9 days after egg laying, and adults emerge
about 5 days after pupariation.

The neurons of labellar taste sensilla arise within the
first 32 h after pupariation (Lakes and Pollack, 1990). Neuronal birth time was determined by labeling cells in
S-phase with the thymidine analog 5-bromo-2'-deoxyur-
idine (BrdU). BrdU (Sigma: 1-2 µl of a 50 mM solution
in a 1:1 mixture of Culiphora saline (Normann and
Duve, 1969) and GIBCO antibiotic-antimycotic mixture) was injected into either the abdomen or the head of
pupae, either within 30 min of pupariation (designated 0
h), or 4, 8, 12, 16, 20, or 24 h after pupariation. The time
of pupariation was marked by the transformation of the
larva into a white prepupa (Zdarek and Fraenkel, 1972).
Pupae were permitted to develop to the age 72 h after
pupariation, at which time they were either dissected im-
mediately or held at 4°C for up to 2 days before dissec-
tion (during which time little or no further development
occurred).

To study the general disposition and time of appear-
ance of taste sensilla, neurons were labeled with anti-
orseradish peroxidase (anti-HRP; Cappel), which
binds to the surfaces of insect neurons (Jan and Jan,
1982). Labella were dissected from developing pupae
and fixed in Zamboni’s fixative (>1 h), incubated for 1 h
in 0.1 M phosphate buffer (pH 7.3) containing 0.3%
Figure 2: Differentiating taste sensilla, labeled with anti-HRP. (A) At 30 hours after pupariation (ap), developing papillae. The single arrow indicates a papilla with two cells, which are beginning to form apical processes. The twin arrows indicate the two cells of another papilla, one of which is stained only lightly. (B) At 21.5 hours ap, developing papillae. The arrow indicates a sensillum with only two cells at this stage. (C) At 22.5 hours ap, developing lateral papillae. The indicated sensillum has three darkly staining cells (probably neurons) and two additional cells, situated apically and marked by arrows, that stain lightly; these may be accessory cells. (D) At 21.5 hours ap, developing taste hairs. The arrows indicate two apical, lightly
Triton X-100 (PBT) and 10% nonimmune goat serum (NIGS), and incubated for 16–20 h in PBT containing 1% NIGS and rabbit anti-HRP at a dilution of 1:800 to 1:1600. Bound antibody was visualized with the peroxidase-antiperoxidase (PAP) procedure. Following incubation in anti-HRP, tissue was incubated in goat anti-rabbit immunoglobulin G (IgG) (GAR, 1:20) followed by rabbit-PAP rabbit immunoglobulin G (IgG) (GAR, 1:200) followed by PBT containing 1% NIGS and rabbit anti-HRP (1:800), and mouse anti-BrdU (Becton-Dickinson; I:100) for 16–20 h. Tissue was then incubated in a reaction mixture consisting of 0.025% diaminobenzidine (Sigma) and 0.5% hydrogen peroxide in 0.05 M Tris buffer (pH 7.6). The reaction was stopped when neurons became apparent under a dissecting microscope, usually after 4–8 min, by rinsing the tissue with cold buffer. Specimens were dehydrated in an alcohol series, cleared in methyl salicylate, and mounted in either Permount or Canada balsam.

BrdU-labeled neurons were visualized with double-label immunocytochemistry. Labela were fixed (>1 h) in Carnoy’s I, incubated in 2 M HCl (10 min), incubated in 10% NIGS in PBT followed by PBT containing 1% NIGS, rabbit anti-HRP (1:800), and mouse anti-BrdU (Becton-Dickinson; I:100) for 16–20 h. Tissue was then incubated for 1 h in a mixture containing lissamine rhodamine-tagged GAR (1:100) and fluorescein-tagged goat anti-mouse IgG (GAM, I:100; both secondary antibodies from Jackson ImmunoResearch Laboratories; in some experiments rhodamine-tagged GAM and fluorescein-tagged GAR were used instead). Preparations were mounted in a mixture of 10% phosphate buffer, pH 8.6, and 90% glycerol, containing 4% n-propyl gallate (Giloh and Sedat, 1982), and viewed both with standard fluorescence microscopy and with a Leica confocal laser scanning microscope.

All incubations were followed by repeated washes in buffer. Incubations and washes were done at room temperature except for the 16–20 h incubations, which were at 4°C.

The qualitative conclusions are based on observations of more than 100 preparations. The quantitative data, presented in the tables and in Figures 6 and 7, however, are based on only a few preparations per injection time. Counting the numbers of neurons and BrdU-labeled nuclei in sensilla was a laborious process that entailed making series of confocal optical sections through overlapping small regions of the labellum at relatively high magnification and required approximately 10 hours per labellum. Consequently, the number of preparations analyzed in this way was necessarily small.

RESULTS

Disposition of Taste Sensilla and Their Neuronal Complements

Figure 1 (A) illustrates neurons of taste sensilla of a 72 h pupa. Although the pupal period is only 60% completed by this time, and cuticular features of the sensilla continue to differentiate, all of the cellular components of the sensilla are already present (De Kramer and Van Der Molen, 1984; Lakes and Pollack, 1990). The development of the antibody-impermeant cuticle interferes with labeling at later stages. In Figure 1 (A) the developing labellum has been opened and flattened so that the dorsolateral margin of the lobe surrounds the oral surface. The taste hairs densely populate the dorsolateral margin, whereas the papillae are more sparsely distributed on the oral surface. Each lobe has, on average, approximately 125 taste hairs and 68 papillae (of which approximately 28 are lateral papillae and 40 are interpseudotracheal papillae, see later) (Wilczek, 1967).

The taste hairs of 72-h pupae [Fig. 1 (A), h] characteristically have five neurons each [Fig. 1 (B)], although several have only four and a few have only three (Table 1). Two sorts of taste papillae can be distinguished. A single row of lateral papillae occurs near the lateral margin of the oral surface, with one papilla at the end of each pseudotrachea [Fig. 1 (A), 1p]. These sensilla generally have three neurons [Table 2; Fig. 1 (C)]. The remaining, interpseudotracheal papillae [Fig. 1 (A), 1p], which occur more medially, most often have four neurons each [Table 3; Fig. 1 (C)].

We did not identify sensilla as individuals that could be recognized from fly to fly, and, consequently, we do not know whether the variability within each of the three sensillum types reflects the existence of subsets of sensilla that consistently have different neuronal complements [as is known to be the case for taste hairs of Drosophila (Nayak and Singh, 1983)], or whether, instead, it results from developmental variability.
Differentiation of a Sensillum

The differentiation of a sensillum, as revealed by anti-HRP labeling, is similar for hairs and papillae. The first sign of appearance of a sensillum is generally weak staining of one or two cells in the epithelium [Fig. 2 (A,B)]. The fact that processes, which eventually form dendrites, can often be seen to grow out of cells at this stage [Fig. 2 (A)] suggests that these cells may be developing neurons. How-
Birth Times of Taste Sensilla

Times of Appearance of Sensilla

Taste neurons can first be detected with anti-HRP at about 8 hours after pupariation. These are neurons of taste hairs. Additional taste hair neurons appear over the next 24 hours, and by about 32 hours the adult complement is attained. These sensilla do not arise in any particular spatial order; new sensilla arise interspersed with older ones [Fig. 3 (A)].

The first taste papillae can be detected with anti-HRP slightly later, at 10 to 12 hours after pupariation. These are the lateral papillae situated midway along the longitudinal axis of the labellar lobe. Later papillae appear over the next 20 hours in a clear spatial pattern, defining a wave of differentiation that spreads anteriorly and posteriorly, as well as medially, from the point at which the oldest papillae arise [Fig. 3 (B,C)].

The times of appearance of sensilla were also determined by BrdU uptake. This is somewhat problematic, because the dynamics of the availability of BrdU following injection is not precisely known. In *Drosophila*, it has been estimated that injected BrdU remains available for uptake for less than 4 hours (Hartenstein and Posakony, 1989). BrdU doses in the present experiment were substantially higher than those used for *Drosophila*, however, and thus the clearance time may be longer. Moreover, BrdU incorporated by cells early in a lineage will be inherited by their progeny (although it will be diluted in the process). The effective duration over which BrdU can label neurons of taste sensilla can be estimated from the spatial pattern of labeling of papillae. This pattern generally confirms the findings from anti-HRP staining. Thus, early injections principally label lateral pa-
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Figure 5  Spatial organization of birth times of papillae. Papillae with no BrdU-labeled neurons are indicated by open circles, those with at least one BrdU-labeled neuron, by filled circles. Times of BrdU injection are indicated. Because the delicate labellae were sometimes damaged during dissection, and because papillae were sometimes obscured by overlaid tissue, it was seldom possible to score all the papillae of a single labellar lobe. Thus, the drawings show less than the full complement of papillae.

pillae located midway along the long axis of the labellum. Later injection times label successively anterior and posterior lateral papillae, as well as more medial papillae [Fig. 4(A), 5]. BrdU-labeled taste hairs, in contrast, are intermixed with unlabeled hairs [Fig. 4(B)], thus confirming the absence of a spatial organization of birth times for hairs. Papillae in a given region can be labeled by BrdU injections separated by 4 to 8 hours (Fig. 5), suggesting that BrdU is available to label cells, either through direct uptake or through inheritance, for at least this time period. Thus, the temporal resolution is rather low.

Nevertheless, BrdU injections yield a picture of the timing of sensillar appearance similar to that obtained with anti-HRP staining (Fig. 6). Substantial numbers of hairs are labeled by injections at time 0. There is some indication that hairs may be born in two waves, the first labeled most frequently by early BrdU injection, and the second with peak labeling at 12 hours. Early injections label only small numbers of papillae. The lateral papillae, which are the first to appear with anti-HRP staining, label most frequently with BrdU injected at 8 hours, and the interpseudotracheal papillae are most often labeled by injection at 12 hours.

Effects of BrdU on Sensillum Development

Visualization of BrdU-labeled neurons with fluorescent secondary antibodies (which was necessary for confocal microscopy) required high BrdU doses. Probably as a consequence of this, BrdU injection affected development of sensilla. This was evident as increased numbers of sensilla with atypical numbers of neurons (Tables 1–3). Thus, although 91% of hairs in control 72 hour pupae, which did not receive BrdU injections, had five neurons, only 82% of hairs in BrdU-injected pupae had five neurons ($p < 0.01$, chi square; data for all injection times were pooled). Similar results were
Birth Times of Taste Sensilla

obtained for taste papillae (lateral papillae: 86% had three neurons in control pupae, 72% in BrdU-injected pupae, \( p < 0.001 \); interpseudotracheal papillae: 82% had four neurons in control pupae, 68% in BrdU-injected pupae, \( p < 0.001 \)). Although a few of the atypical sensilla had more than the normal number of cells, the main effect of BrdU was to decrease cell counts.

Age Relationships of Neurons within a Sensillum

Figure 7 depicts the distributions of sensilla with various numbers of labeled neurons, pooled for all injection times. The aim of this analysis is to gain insight into the patterns of mitosis that give rise to the neurons of a sensillum. Because these patterns may be aberrant in sensilla with atypical cell numbers, these sensilla have been excluded from the analysis. Thus, only five-celled taste hairs, four-celled interpseudotracheal papillae, and three-celled lateral papillae are included in Figure 7.

There was a strong tendency for the neurons of a sensillum to be labeled with BrdU in an all-or-none fashion, i.e., most often either all of the neurons of a single sensillum were labeled with more or less equal intensity or none was labeled. Only rarely did either the leading or trailing edge of the BrdU pulse fall within the lineage of a sensillum, thereby either labeling some neurons but not others or labeling different groups of neurons with different intensity (Fig. 8).

Among these heterogeneously labeled sensilla, there were clear tendencies toward particular combinations of labeled and unlabeled cells (Fig. 7). In all 17 instances of differentially labeled interpseudotracheal papillae, two neurons were labeled (or labeled more strongly) and two were unlabeled (or labeled more weakly); in no case was the distribution 1:3 or 3:1. Similarly, among differentially labeled taste hairs, there was a strong preference for a 2:3 or 3:2 distribution \( (n = 19 \text{ and } 20, \text{ respectively}) \); there were no cases with only one of the five neurons labeled, and only a single case with four of five labeled. The differentially labeled lateral papillae, of course, had either one or two labeled neurons \( (n = 5 \text{ and } 7, \text{ respectively}) \).

Accessory Cells

The number of nonneuronal accessory cells contributing to each sensillum is a matter of some uncertainty (see Discussion). Most cuticular sensilla of flies include at least three accessory cells: the trichogen and tormogen cells, and the thecogen cell. The antibody used to visualize neurons in the present study, anti-HRP, does not label these cells in mature sensilla. Nevertheless, the presence (or absence) of BrdU-labeled nuclei, which, because of their positions, are good candidates for belonging to accessory cells, can be informative about the developmental relationships of accessory cells and neurons. Because of the dense packing of taste hairs, it was often difficult to assign these putative accessory cell nuclei to one specific sensillum or another. The following is thus based on observations of favorable (i.e., well-isolated) examples of taste papillae.

Papillae were often seen with nearby BrdU-labeled nuclei that possibly belonged to nonneuronal accessory cells. A pair of nuclei was often seen near the bundle of dendrites that exit from the apical end of the neuronal cluster; these are good candidates for the nuclei of the trichogen and tormogen cells [Fig. 9(A,B)]. There was a tendency for these nuclei to be labeled more often in younger sensilla (i.e., in those papillae on the medial edge of the group with labeled neuronal nuclei). Often, a labeled nucleus was found closely apposed to the group of neuronal somata; this is a good candidate for the thecogen cell [Fig. 9(B,C)]. No clear age-related pattern of labeling of this nucleus was detected, and labeled, putative thecogen cell nuclei were associated with papillae both with and without labeled nuclei of putative trichogen and tormogen cells. Often, no BrdU-labeled nonneuronal nuclei were found in the immediate neighborhood of BrdU-labeled neurons [Fig. 9(D)].
DISCUSSION

Effects of BrdU on Development

The high dose of BrdU that was required for visualization of labeled nuclei with fluorescent secondary antibodies resulted in an increased proportion of sensilla with abnormal neuron counts; generally, a larger proportion of sensilla had fewer than normal cells (Tables 1–3). Truman and Bate (1988) noted that high BrdU doses caused Drosophila larvae to grow more slowly and resulted in sickly adults. In the present work, the largest effects on cell count generally occurred for injection times that labeled the largest proportions of sensilla (compare Tables 1–3 with Fig. 6). This suggests that BrdU did not cause decreased cell counts through some sort of general toxicity, but rather that its deleterious effects depend on incorporation into DNA.

Incorporation of BrdU is known to have a number of effects on cells, including lowered rate of cell division in cultured cells (Kajiwara and Mueller, 1964), chromosome breakage and other cytogenetic aberrations (Hsu and Somers, 1961), interference with DNA-protein interactions (Lapeyre and Bekhor, 1974), and a variety of defects in cellular differentiation (Rutter et al., 1973). It seems likely that the high BrdU dose used in the present work either interfered with the viability of some cells or altered their differentiation or affected their ability to express the molecules recognized by anti-HRP.

Even though BrdU had clear effects on cell counts, the modal counts for injected pupae were the same as those for controls in nearly all experiments (the single exception being counts for lateral papillae in pupae injected at 8 hours). Because only sensilla with these modal values were included in the analysis of distributions of labeled and unlabeled neurons per sensillum, it is unlikely that the main conclusions were affected by the deleterious effects of high BrdU doses.

Spatial Organization of Birth Times

We did not detect any clear spatial organization of taste hairs of differing ages. Although there is some...
Figure 8  Heterogeneous labeling of sensilla. (A and B) Different confocal optical sections through the neurons of the same taste hair, in a preparation injected with BrdU at 0 hour after pupariation (ap). Only two of the four neurons in A have BrdU-containing nuclei; the fifth neuron, shown in B, is unlabeled. (C) An interpseudotracheal papilla, with two strongly labeled nuclei and two that are labeled weakly. Image is a reconstruction of 20 optical sections spaced 1 μm apart.

indication that taste hairs may arise in two waves, one near the onset of pupariation and the other about 12 hours later, neither of these waves is restricted to a particular region of the labellum; no matter when pupae were injected with BrdU, labeled and unlabeled hairs were intermixed.

In Drosophila, Ray et al. (1993) found that taste hairs are born in three waves, with the oldest hairs being, in general, situated more laterally, and younger hairs more medially. Phormia has about four times as many labellar taste hairs as Drosophila. This numerical complexity, together with the relatively poor temporal resolution of BrdU labeling, may have obscured any spatial pattern of the timing of hair differentiation.

For the taste papillae, however, such a pattern is clear; the oldest papillae are the most lateral ones and are situated midway along the anterioposterior axis; younger papillae appear in a wave that radiates from this point medially as well as anteriorly and posteriorly. The spatial pattern of papillar differentiation in Phormia is thus similar to the pattern of hair differentiation in Drosophila.

Developmental Relationships among the Neurons of a Sensillum

The distributions of BrdU-labeled neurons per sensillum (Fig. 7) argue strongly against the possibility that the neurons are produced by an iterated series of asymmetrical divisions (the stem cell model). If that were the case, then, because sensilla arose throughout the 24 hours that were probed with BrdU injections (Fig. 6), the distributions of BrdU-labeled neurons per sensillum should in-
Figure 9 (A) Confocal optical section through an interpseudotracheal papilla showing two additional BrdU-labeled nuclei at the apical end of the cell cluster. (B) Optical section through another interpseudotracheal papilla, showing two Brdu-labeled nuclei (one of which is only faintly visible in this section) associated with the dendrites and, in addition, a third labeled nucleus near the cluster of neuronal somata. (C) Optical section through a third interpseudotracheal papilla, which had a BrdU-labeled nucleus associated with the somata, but did not have a pair of nuclei at the apical end of the cell cluster. (D) An interpseudotracheal papilla with four Brdu-Labeled neurons, but no other labeled nuclei in the immediate vicinity. Image is a reconstruction of 25, 1 μm confocal optical sections. A–C are from preparations injected with BrdU at 12 hours ap, D was injected at 16 hours ap.

include all possible values. However, only certain combinations were observed (Fig. 7).

Ray et al. (1993) used their observations of BrdU labeling, supplemented by counts of the numbers of cells in a developing sensillum as a function of time, to derive the developmental relationships among the cells of a sensillum. According to their model [which is reproduced, in modified form, in Fig. 10(A)], one of the daughters of the sensory mother cell, the “neurogenic” precursor, produces, at its first division, two second-generation precursors. One of these divides to produce the thecogen cell and one of the neurons, and the other undergoes two more rounds of division, the first producing two third-generation precursors, and the second producing, from these, the remaining four neurons. Depending on the time of BrdU injection, Ray et al. (1993) found that either zero, four, or five neurons were labeled, the cases with four neurons labeled being those in which BrdU was not incorporated by the precursor of the thecogen cell (which was also unlabeled) and its sister neuron.

The distribution of labeled neurons per taste...
hair observed in the present study differs from that observed by Ray et al. (1993) in Drosophila. We found that either zero, two, three, or five neurons were labeled, with the exception of a single case in which four neurons were labeled (Fig. 7). Injections were done by Ray et al. at chosen times in the development of particular identified sensilla, whereas in the present experiments sensilla were not identified as individuals, and injection at any given time caught different sensilla at different stages of development. However, this difference in procedure cannot explain the difference in labeling distributions.

The present data are not necessarily incompatible with the lineage proposed by Ray et al. (1993); they could be generated, for example, if the S phase of one of the third-generation precursors, i.e., one of the cells that divides to produce two neurons, were delayed with respect to the other third-generation precursor. In addition, the S phase of one of these precursors would have to be synchronous (at least within the temporal resolution of our experiment) with that of the precursor that gives rise to the thecogen cell and its sister, the remaining neuron [Fig. 10 (A)]. Although other models can be devised that give rise to the present BrdU-labeling data without requiring asynchronous divisions of the two third-generation precursors [an example is given in Fig. 10 (B)], these are not compatible with counts of the numbers of cells by Ray et al. in developing sensilla as a function of time. Because the taste hairs of Phormia and Drosophila are similar in many respects, including neuron number, neuronal phenotypes (Falk et al., 1976; Fujishiro et al., 1984), anatomy of central projections (Nayak and Singh, 1985; Yetman and Pollack, 1986), and role in behavior (Falk and Atidia, 1975; Rodrigues and Siddiqi, 1978), we assume tentatively that they are also similar in development, and we therefore favor the model drawn in Figure 10 (A).

Our observations suggest strongly that the four neurons of an interpseudotracheal papilla comprise two pairs of siblings. In all differentially labeled, four-celled interpseudotracheal papillae, the split between labeled and unlabeled neurons was 2:2.

Morphologically, interpseudotracheal papillae are extremely reduced hairs (Peters, 1963). Neurophysiologically, at least some of the neurons of papillae are similar in response characteristics to those of taste hairs (Dethier and Hanson, 1965). Thus, like the hairs, the papillae have a mechanoreceptor, a sugar receptor, and a salt receptor (as well as a fourth, as yet uncharacterized, receptor). Because of these similarities in structure and in physiology, it seems plausible that the papillae and hairs are also similar in development. One can speculate that a papilla could be constructed by the same lineage proposed by Ray et al. (1993) for taste hairs simply by eliminating, perhaps through cell death, the neuron that is the sibling of the thecogen cell.

Let us assume for the moment that the phenotypically different neurons of a sensillum occupy constant positions within the lineage of the sensillum, i.e., that in the taste hairs the thecogen's sibling is always, for example, the water receptor (or sugar receptor or mechanoreceptor, etc.). Then, if the lineage of the papilla is indeed a modification of that for a hair of the sort just outlined, one can predict that, in hairs, the thecogen's sibling is neither the mechanoreceptor, the sugar receptor, nor the salt receptor, because all of these cell types occur in the papilla. This leaves the water receptor and the poorly characterized fifth cell as the most likely candidates for the thecogen's sibling (providing that the two assumptions made are correct, namely: (1) that phenotype and position within the lineage are strictly correlated, and (2) that the lineage of an interpseudotracheal papilla is an "edited" version of the lineage of a taste hair).

### Accessory Cells

In all sensilla of Drosophila studied thus far, one of the accessory cells (the thecogen cell of external sensilla, the scolopale cell of chordotonal sensilla) is a sibling of a neuron (Bodmer et al., 1989, Ghy-
sen and Dambly-Chaudière, 1993; Ray et al., 1993). A clear conclusion of the present study is that, in interpseudotracheal papillae, none of the neurons is a sibling of an accessory cell. Two pieces of evidence support this. First, the BrdU-labeling distributions indicate that the four neurons comprise two pairs of siblings, i.e., all of the neurons’ siblings are themselves neurons. Second, we observed many cases in which all four neurons of a sensillum had BrdU-labeled nuclei, while there were no other BrdU-labeled nuclei, which might belong to accessory cells, in the vicinity [Fig. 9(D)]. In these cases, all of the accessory cells must have had different birth times from any of the neurons, and thus could not have been their siblings.

Unfortunately, it is not clear how many accessory cells are associated with taste papillae. In Phormia, an early (and, to our knowledge, still the only) ultrastructural study reported only two accessory cells (Larsen, 1962). In Calliphora vicina, the same laboratory has reported both two (Van der Wolk, 1980) and three (Menco and Van der Wolk, 1982) accessory cells. Papillae of the fleshyly Böttcherisca peregrina have three distal accessory cells as well as a more proximal glial cell (Tominaga et al., 1969). Drosophila taste papillae appear to have only one accessory cell and two neurons (Falk et al., 1976). Several markers observable with light microscopy label both neurons and accessory cells in Drosophila; however, none of these was used in the present work. Some of the markers, enhancer-trap lines, are not available for Phormia. Another, the monoclonal antibody 22c10, does not reliably label all of the neurons in Phormia (J. Pak and G. Pollack; unpublished observations), and consequently was not used. BrdU-labeled nuclei were often observed that were good candidates for accessory cells (Fig. 9), but in the absence of independent markers the identities of these cells cannot be known with certainty. It is interesting, nevertheless, that the nuclei found near the apical end of the neuronal cell cluster (the candidates for the trichogen and tormogen cell nuclei) tended to be labeled more frequently in younger papillae. This is the expectation according to the lineages outlined in Figure 10, where these cells are born early in the lineage of the sensillum.

Even for the more extensively studied taste hairs, the number of accessory cells, and their developmental relationships to the neurons, remain somewhat unclear. Ultrastructural investigations in blowflies have reported both two (Hansen and Heumann, 1971) and three (Felt and Vande Berg, 1976; Hansen and Hansen-Delkeskamp, 1982) accessory cells. However, two ultrastructural investigations of Drosophila taste hairs both reported that the dendrites are wrapped by four concentrically arranged accessory cells (Falk et al. 1976; Nayak and Singh, 1983). The lineage scheme proposed by Ray et al. (1993) (who cite both of these articles) and reproduced in the present article [Fig. 10(A)] accommodates only three accessory cells. The identity and origin of the fourth remain unknown.

CONCLUSION

The goal of this study was to gain insight into the sequences of mitoses that generate the groups of neurons of multiply innervated chemosensilla. Because the patterns of BrdU incorporation among the neurons of a single sensillum indicate that subsets of these neurons are siblings, the neurons cannot be generated by an iterated series of asymmetrical divisions (the stem cell model). Also, because different neurons share birth times, their differing phenotypes cannot be determined solely by relative birth times. Instead, as in many other examples (cited in the Introduction), cell–cell interaction is likely to play an important role in the determination of phenotype.

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