INTRODUCTION

Although the taste organs of flies might, at first glance, seem esoteric subjects for neurobiological study, they have in fact played an important role in the development of ideas about how nervous systems (and organisms) function. The essential features of these organs, from which their attraction as experimental objects derives, were worked out 40 years ago (Dethier, 1955). By stimulating single taste hairs with various substances and observing whether these were "accepted" or "rejected" by the fly (i.e., whether they elicited or inhibited proboscis extension), Dethier established that a single taste hair can provide the central nervous system with sufficient information to distinguish between mechanical stimuli, water, sugars, and salts. He concluded that this broad behavioral competence of a single sensillum was due to its innervation by several different sensory neurons, each with its own characteristic stimulus sensitivity, and each eliciting either acceptance or rejection. Although not entirely accurate in detail (we now know that the taste hairs studied by Dethier typically have five neurons rather than the three he initially proposed, and that their response spectra are more complicated than was originally supposed), this work was seminal because it was among the earliest in all of neurobiology to assign clear functions to single, identified neurons. Moreover, Dethier arrived at these early conclusions without the benefit of electrophysiological data; the first recordings of action potentials from taste hairs were reported only in the same year (Hodgson et al., 1955).

In addition to neurons, taste organs include several nonneuronal cells which play important roles in both the development and the function of the organ. The focus of this review will be on the phenotypic specificity of the various component cells of taste organs. The two central questions are: 1) how do the various cells differ from one another, and 2) how do these differences come about during development?

TASTE SENSILLA AND BEHAVIOR

Taste sensilla of flies occur as two principal types: taste hairs (sensilla trichodea), found on the legs, wings, ovipositor, and mouth parts, and taste papillae (sensilla basiconica), found on the oral surface and within the pharynx (Nayak and Singh, 1983; Stocker and Schorderet, 1981; Wilczek, 1967). The functions of the hairs on the ovipositor and wings are not well-understood, though the former seem likely to play a role in the selection of oviposition sites (Merritt and Rice, 1984). Those on the legs and mouth parts, however, are clearly important for feeding. Flies first evaluate their food by stepping in it. If the report of the taste hairs on the legs is acceptable, the fly responds by extending its proboscis. This brings a second group of taste hairs, those on the aboral surfaces of the labellar lobes, into contact with the food. If they too find the stimulus acceptable, the fly opens the labellar lobes and in so doing exposes a third set of receptors, the taste papillae of the oral surface, to the food. Adequate stimulation of the papillae results in ingestion (Dethier, 1955).

In addition to sensing the quality of potential food, the taste hairs also provide information about its location. Stimulating only one leg with sucrose, e.g., causes the fly to pivot towards the stimulated side (Dethier, 1957). The labellar hairs also encode information about stimulus location. Although in a natural situation labellar hairs are not normally stimulated until after the proboscis has already been extended, stimulation of a labellar hair while the proboscis is still retracted can evoke proboscis extension. The response is highly directed, and aims the proboscis along a trajectory determined by the location of the hair that is stimulated, e.g., stimulation of an anterior hair on the
right labellar lobe evokes extension ahead and towards the right (Yetman and Pollack, 1987). Labellar hair location is also reflected in the natural response to stimulation of these sensilla, i.e., spreading apart of the labellar lobes; stimulation of labellar hairs on one side causes only the ipsilateral labellar lobe to open (Pollack, 1977). The positional information provided by labellar hairs may be used, under natural conditions, to position the mouthparts properly with respect to the food source.

**PHENOTYPES OF THE COMPONENT CELLS OF TASTE SENSILLA**

**Physiological Characteristics of Gustatory Neurons**

**Taste Hairs.** Electrophysiological experiments have confirmed the multiplicity and specificity of gustatory neurons. Extracellular recordings are made either by placing a single fluid-filled pipette over the tip of a hair, thus simultaneously stimulating the dendrites of receptors and recording their responses through the hair's pore (Hodgson et al., 1955; Morita et al., 1957), or by placing a recording electrode in one of the hair's lumina and stimulating the receptors with an independent pipette placed over the tip (Morita and Yamashita, 1959). These techniques yield multiunit responses in which the activity of the different neurons can be distinguished on the basis of the sizes and shapes of their action potentials. Because spikes with different characteristics are reliably elicited by different stimulus substances (e.g., in Phormia, NaCl reliably elicits large, biphasic spikes, while stimulation with sugars results in spikes that are smaller and lack a prominent negative component), the various neurons can be identified with reasonable confidence.

The sugar (S) neuron. This is the most extensively studied neuron in the taste hair. It responds vigorously to a variety of sugars, to some amino acids, and to a few other compounds (see below). A major focus has been to identify the number and nature of receptor sites. Experiments in several species point to the existence of separate sites for pyranoses and furanoses. These sites are distin

Taste hairs have been classed into types based on morphological criteria, chiefly size and location (Grabowski and Dethier, 1954; Wilczek, 1967). Comparisons of the relative responses of different labellar hair types of Protophormia showed that the effectiveness of 4-nitrophenyl-alpha-glucoside (4-NP-A-Glc) in eliciting S neuron spikes varied independently of those of furanoses and pyranoses (Wieczorek and Köppl, 1982), thus suggesting the presence of yet another site. This was supported by the observation that addition of 4-NP-A-Glc to saturating concentrations of either glucose (pyranose) or D-fucose (furanose) enhanced the response of the S neuron.

Finally, Amakawa et al. (1992) found, in Phormia, that S neurons respond to the membrane-impermeable cyclic nucleotides, cAMP and cGMP, and proposed that these acted at a distinct site. They showed that addition of cGMP enhanced the response to sucrose but, because the sucrose concentration used was low (15 mM) and certainly nonsaturating, it did not consider the possibility that the nucleotides might act at other, better established, sites on the S neuron (e.g., the amino acid site(s)).

Recently, Ozaki et al. (1993) used affinity gel electrophoresis to isolate membrane-associated, putative pyranose and furanose receptor proteins from extracts of both whole labella and of isolated sensilla of Phormia. The dissociation constants of these proteins for different sugars are consistent with values estimated from S neuron responses. Further confirmation of their identity as receptors could come from their amino acid sequences, if it is possible to purify sufficient amounts of protein for sequencing studies.

The water (W) neuron. As its name suggests, the W cell responds to water. The mechanism by which it does so is unclear. Rees (1970) proposed electrokinetic streaming as the mechanism for generation of a receptor potential in response to water; essentially, osmotic forces drive water into the cell through channels in the membrane, with cations being carried in passively, causing the cell to depolarize. Consistent with this, the activity of the W cell is suppressed by a variety of solutes which are not obviously related except in that they increase the osmolarity of the stimulus (Dethier, 1976; Evans and Mellon, 1962). Wieczorek (1980), however, has argued that electrokinetic streaming is unlikely to be able to generate potentials as large as the recorded receptor potential to water. Further, Wieczorek et al. (1988) showed that pronase treatment specifically suppresses the response of the W neuron to water, while leaving its response to sugar (see below) unaffected, thus implicating specific proteins in the response.

The W neuron also responds to sugars. Wieczorek and Köppl (1978) and Wieczorek (1980) showed in Protophormia that the W neuron was activated specifically by furanose sugars, with sensitivity to different furanoses paralleling that of the S neurons. In Drosophila, the W neuron is normally inhibited by sucrose. However, in the gust A mutant, in which the S neuron's
response to pyranoses is depressed, sucrose (which acts at the pyranose site) fails to inhibit the W neuron (Rodrigues and Siddiqi, 1981). Thus, inhibition of the W neuron by sucrose is not simply an osmotic effect, but rather appears to be mediated by a specific pyranose site on the W cell.

The “classical” salt receptor (L1) neuron. The best stimuli for the L1 cell are the monovalent alkali halides (Gillary, 1966). In Phormia and Calliphora there is a hierarchy of effectiveness for both the cation and anion components of the salts, which differs between the two species and even according to hair type within a species (Dethier, 1976). Mixing and cross-adaptation experiments, together with similarities in the dose-response curves, suggest that all the alkali halides act in a similar manner on L1 and possibly share a single receptor site (Gillary, 1966).

In Drosophila, the response of L1 is largely unaffected by the anion (Arora, 1985). Cross-adaptation experiments suggest that there are two cationic acceptor sites on L1, a Na⁺-specific site and a nonspecific cationic site. This hypothesis is supported by genetic evidence; in the mutant gust E, L1’s response to Na⁺ is reduced, but its response to K⁺ is normal (Siddiqi et al., 1989). It is not yet clear whether the two cation sites constitute a special class of “ion receptors” or whether they are in fact ion channels, which could, in theory, directly mediate salt reception/transduction. There is some evidence for the latter possibility from studies in vertebrates (Kinnamon, 1988).

In Protophormia, Schnuch and Hansen (1990) found an increase in the frequency of salt receptor spikes upon addition of sugars to low concentrations of NaCl, with pyranose sugars showing a stronger effect than furanoses. Thus, it seems possible that the “salt” neuron may also carry receptor sites for sugars.

The “fifth” cell (L2). Also called the “anion receptor,” this is the least understood of the four chemosensory neurons. The cell exhibits a high-threshold response to NaCl, but its firing frequency is characteristically low and does not correlate well with increase in stimulus concentration, suggesting that NaCl is not its best stimulus (Dethier, 1976). Dethier and Hanson (1968) showed that the fifth cell of Phormia responds to sodium salts of fatty acids, with the response being a function of the carbon chain length. Studies on tarsal sensilla of Phormia (McCutchan, 1969a,b) revealed that this cell responds more strongly to acids than to salts. In Drosophila, too, there is evidence that acids are better stimuli than NaCl for L2 (Arora, 1985).

The mechanoreceptor. Wolbarsht and Dethier (1958) provided physiological evidence for a mechanosensory neuron in Phormia taste hairs. They recorded a distinct spike in response to deflection of the hair, which persisted after amputation of the tip of the hair and after adaptation of the chemosensory neurons. Ultrastructural studies (Falk et al., 1976; Nayak and Singh, 1983; van der Wolk, 1980) reveal that one of the neurons of the hair terminates at its base, and includes a tubular body, a structure that is found in the dendrites of neurons that innervate mechanosensory hairs (McIver, 1985). Thus, the mechanosensory neuron of taste hairs appears to be a standard tactile receptor.

Tarsal Receptors. Studies on Phormia indicated that tarsal sensilla are similar to those on the labellum in that they possess a sugar neuron, a “classical” salt receptor, a “fifth” cell responsive to high concentrations of NaCl and to acids, and a mechanoreceptor (Dethier and Hanson, 1968). However, there are consistent differences among the responses of different hair types, and also between specific hairs of a given type, in terms of thresholds, dose-response curves, and reaction spectra (McCutchan, 1969a; Shiraishi and Tanabe, 1974). Although behavioral experiments indicated the presence of a water receptor cell, this could not be confirmed electrophysiologically in Phormia. From an intensive study of terminal tarsal hairs of Calliphora, van der Starre (1972) concluded that two, or sometimes three, neurons of a sensillum responded to water. He was unable to detect specific S and W neurons, since the same two neurons seemed to respond to both sugar and water stimuli. Thus, tarsal sensilla, though broadly similar to their labellar counterparts, exhibit differences in response as a function of hair type or position.

Taste Papillae. In Phormia (the only fly for which physiological data on papillae exist), there are two distinct classes of papillae on the oral surface of the labellum: the lateral papillae, with three neurons each, and the interpseudotracheal papillae, which have four neurons each (Pollack and Lakes-Harlan, 1995). Recordings from the latter demonstrate the existence of a mechanoreceptor, a sugar receptor, and a salt receptor; the properties of the fourth cell are as yet unknown (Dethier and Hanson, 1965). In its general properties, the papillary S neuron is similar to that of labellar hairs, i.e., the relative stimulating effectiveness of different sugars is largely similar for the two sensillum types. However, S cells in the two sensilla are not identical, e.g., papillae, but not hairs, are sensitive to sorbitol. Responses of papillae to salt are more complicated than those of hairs, with two neurons, rather than only one, usually responding.

Papillae of Drosophila have only two neurons. Morphological evidence indicates that one of these is a mechanoreceptor and the other is a chemoreceptor (Nayak and Singh, 1983). The specificity of the chemoreceptor is not known.

Nonneuronal Components of Taste Hairs. Although the nonneuronal cells of insect sensilla are named for their developmental roles (trichogen, hair-forming; tormogen, socket-forming; thecogen, sheath-forming), they are also important for the functioning of the mature sensillum (Morita, 1992; Thurm and Küppers, 1980). In taste hairs, as in other sensillum types, the apical membranes of the trichogen and tormogen cells define the receptor lymph cavity, a region that is structurally, chemically, and electrically isolated, by septate junctions, from the haemolymph space (Felt and Vande Berg, 1976). The outer segments of the dendrites of the receptor neurons, clad in their dendritic sheaths, are situated in the receptor lymph cavity and are bathed in its contents. The membranes of the trichogen and tormogen cells are highly folded, bear a large number of particles on their cytoplasmic surfaces, and are associated with large numbers of mitochondria (Felt and Vande Berg, 1976; Menco and van der Wolk, 1982); thus, their morphological features suggest a high level of metabolic activity. Electrical and chemical measurements demonstrate that these cells pump potassium into the receptor lymph cavity and by so doing
create a transepithelial voltage of up to 100 mV, with the receptor lymph cavity positive with respect to the haemolymph (reviewed in Thurm and Kuppers, 1980). When transducer channels in the outer dendritic membrane open, as a result of sensory stimulation, the transepithelial voltage is partly shunted through the receptor neuron. Thus, the transepithelial voltage established by the trichogen and tormogen cells contributes to the receptor potential elicited by stimulation (Morita, 1992), augmenting it by up to 100% (Thurm and Kuppers, 1980).

The accessory cells may also play a role in regulating the access of stimulating chemicals to the dendrites of chemoreceptor neurons. Long-term stimulation of a taste hair results in variations in spike rate that are correlated with variations in electrical resistance (de Kramer and van der Molen, 1980; Sturckow, 1971), suggesting that the access of stimulants to the dendrites (reflected as variations in resistance) may be regulated. A viscous material, which may be secreted by one or more of the accessory cells, can often be observed at the tips of taste hairs (Sturckow, 1967), and may serve to restrict entry of stimulating substances through the terminal pore. In addition, the terminal pore appears to be able to adopt a variety of morphological states, ranging from wide open to closed (Sturckow et al., 1973). Although the mechanism for adjusting the diameter of the pore is not clear (though it may be related to pressure within the lumen of the hair: Sturckow et al., 1973), implication of accessory cells seems possible.

In addition to the trichogen, tormogen, and thecogen cells, sensilla usually include a glial cell which enfolds the basal portion of the somata group and the initial portions of their axons (Felt and Vande Berg, 1976; Menco and van der Wolk, 1982; Tominaga et al., 1969).

Comments and Cautions

Interspecies comparisons. The foregoing descriptions of the properties of gustatory neurons are drawn from studies on a number of species. Although gross interspecies similarities clearly exist (e.g., the existence of mechano-, water, sugar, and two salt receptors), it is unrealistic to expect these parallels to extend to the finest levels of organization, e.g., the affinities and molecular structures of receptor molecules. Presumably the characteristics of the various receptors in each species have been shaped by the selective pressures imposed by their own feeding habits and lifestyles.

Intersensillum comparisons. Interpretation of the functional roles of the various receptor neurons depends on comparisons of behavioral and electrophysiological results. The same comparisons are utilized to isolate and characterize mutations that affect sensory responses. Except for a few studies with larger flies, most electrophysiological data are from labellar taste hairs (and, usually, only a subset of those), while most behavioral tests depend either on input from tarsal receptors (e.g., tarsally elicited proboscis extension) or on complex, ill-defined input from a large number of receptors (feeding assays). Because even the scant comparative data that are available indicate that response properties differ between different sensilla, these comparisons of behavioral and electrophysiological results must be made with caution.

Projections of Gustatory Neurons Into the Central Nervous System

Labellar Sensilla

Drosophila. Stocker and Schorderet (1981) first stained the neurons of labellar taste hairs by immersing the entire labellum in cobalt chloride. For reasons that are poorly understood, this treatment stains the taste hairs selectively, sparing the taste papillae. This technique revealed that the taste hairs project to several discrete regions in the anterior subesophageal ganglion. When the labellum was crushed prior to immersion in cobalt, an additional, more ventral projection was revealed, which may represent the terminations of neurons from the taste papillae. Nayak and Singh (1985) studied the central projections of taste receptors using a modified Golgi procedure; they applied AgNO₃ locally to small regions of the labellum by making holes or cuts in the cuticle before immersing the labellum in the solution. They identified seven neuron types, which they distinguished based on the volume of the terminal arbor, the shape or texture of the arbor, and the region of termination (Fig. 1). They proposed that these seven morphological types might correspond to the seven chemoreceptor neuron types.
that they had previously noted in electronmicroscopic studies (Nayak and Singh, 1983), namely the four chemoreceptors of taste hairs innervated by five neurons (the fifth neuron being a mechanoreceptor), the two chemoreceptors associated with a different population of taste hairs, these innervated by three neurons each (with the third neuron again a mechanoreceptor), and the single chemoreceptor associated with the taste papillae on the oral surface (which also contain a mechanoreceptor). This hypothesis, while intriguing, fails to address two major points: 1) it does not account for why the seven morphological types should represent only chemoreceptors, even though each of the contributing sensillum types includes a mechanoreceptor neuron as well; and 2) it does not allow for variations in projection pattern that might be related to the peripheral position of the receptor cells. Such position-dependent effects are well-established in sensory systems of insects in general (e.g., Murphey, 1981; Pflüger et al., 1988), including the taste system of Phormia (see below).

More recently, Shanbhag and Singh (1992) stained the neurons of an individual identified taste hair by applying horseradish peroxidase (HRP) to the hair after cutting off its tip. The neurons stained with this technique fell mainly into 4 of the 7 morphological types that had been described on the basis of Golgi preparations (Nayak and Singh, 1985). Shanbhag and Singh (1992) dissolved the HRP either in water or in 0.1 M solutions of NaCl, KCl, or sucrose. Interestingly, the particular morphological types of neurons that were stained depended on the solution in which HRP was dissolved. When the HRP was dissolved in 0.1 M NaCl or 0.1 M sucrose, neuron types II and IV (of Nayak and Singh, 1985) were stained most often. HRP in 0.1 M KCl led to frequent staining of types II and VI, and when the HRP was dissolved in distilled water, types I and II were stained. One possible explanation for these results is that inclusion of stimulants with the HRP evoked differential activity in the chemoreceptor neurons, and that these differences in activity resulted in differences in stainability. Water and sucrose stimulate, respectively, the W and S neurons of Drosophila labellar hairs; NaCl and KCl stimulate the L1 receptor at low concentrations (including 0.1 M), and both L1 and L2 at higher concentrations (Rodrigues and Siddiqi, 1978; Siddiqi et al., 1989). One might suppose, then, that type II neurons (which were stained by all of the HRP solutions) might be mechanoreceptors, type IV (stained by both NaCl and sucrose) might include two physiological types (namely the sugar and the L1 salt receptors) which might be similar morphologically (see discussion of projections from Phormia taste hairs, below), and type I might represent the water receptor. The selective staining of type VI neurons by 0.1 M KCl, however, is difficult to reconcile with this hypothesis. If neuronal types I, II, and IV account for the water, mechanos-, sugar, and L1 receptors, then the only candidate left for type VI is the L2 receptor. However, 0.1 M KCl, like 0.1 M NaCl, stimulates mainly the L1 neuron; the L2 neuron is activated only at higher concentrations, and even then it does not distinguish between these two salts (Siddiqi et al., 1989). The hypothesis of activity-dependent staining also depends on the maintenance of selectivity of the chemoreceptors after removal of the tips of the hairs. Although Shanbagh and Singh (1992) provide some evidence for this, more would be welcome, particularly since Dethier (1955) found, for Phormia, that amputation of the hair tip abolished behavioral sensitivity to that hair, at least to sugar and water stimuli. An alternative interpretation of selective staining is that different solutes might differentially affect dendrite diameter, rates of membrane turnover, or other parameters of the neurons, thus leading to differences in their stainability. One way to distinguish between these two sorts of explanation would be to see whether selective staining persists when neural activity is prevented, either by the addition of appropriate pharmacological agents (e.g., tetrodotoxin; Kijima et al., 1988) or by the use of conditional channel mutants, in which neural activity is silenced at restrictive temperatures (Wu and Ganetzky, 1980).

Phormia. Labellar projections have also been studied in Phormia regina (Fig. 2). Yetman and Pollack (1986) stained taste neurons by applying cobaltous lysine to intact taste hairs. As in Drosophila, the projections were restricted to the anterior subesophageal ganglion. Yetman and Pollack (1986) found up to three different projection types among the cells of a single hair. One cell type, the DBPC (dorsalmost bilaterally projecting cell), was found in all 11 identified hairs that they investigated (the “largest” hairs of Wilczek, 1967). The remaining cells showed position-dependent variations in projection patterns. The 11 hairs fell into three groups, composed respectively of anterior, midlevel and posterior hairs. Within each group, the projections of the different hairs were similar. The main distinguishing feature among the three groups was whether the cells terminated ipsilaterally or bilaterally. The DBPC had bilateral branches for all hairs but, for the remaining cells, only the anterior and posterior groups branched bilaterally. Yetman and Pollack (1986) speculated that the presence or absence of bilateral branching might be related to the degree of bilateral symmetry in the motor responses resulting from stimulating the hairs. Because anterior and posterior hairs evoke proboscis extensions that are directed close to the midline, while midlevel hairs evoke more laterally directed extensions (Yetman and Pollack, 1987), the activation of motor circuitry would be expected to be more symmetrical in the former cases than in the latter. One way to promote symmetrical motor activity would be to distribute sensory input symmetrically, via bilateral branching of sensory neurons.

Edgecomb and Murdock (1992) also studied the projections of labellar hairs in Phormia. Their findings were largely similar to those of Yetman and Pollack (1986). However, perhaps because they used slightly different techniques, they revealed an additional cell type (Fig. 3). This cell has a larger-diameter axon and arborizes more centrally in the subesophageal neuropil than the thinner axons. By analogy with the projection of the mechanoreceptor cell from tarsal taste hairs (see below), this is a good candidate for the mechanoreceptor cell.

Despite the marked similarities in physiology and function of the labellar receptors in Phormia and Drosophila, their terminal arbors are not strikingly similar. Several of the cells innervating the single hair studied by Shanbagh and Singh (1992) had bilateral
Fig. 2. Central projections from labellar taste hairs in Phormia. Identities of hairs that were stained are indicated. Three different projection types were observed, from anterior hairs (Hairs 1 and 2, A, B), midlevel hairs (Hairs 3–8, C–E), and posterior hairs (Hairs 9–11, F) respectively. One cell type, the DBPC, was common to all hairs. D: A parasagittal section: A, P, D, and V indicate anterior, posterior, dorsal, and ventral; arrow indicates a posterior tuft of branches that was often seen for midlevel hairs. All other figures are whole mounts viewed in the frontal plane. LB, DB, VB, and PB indicate lateral, dorsal, ventral, and posterior branches. Scale bar in A = 100 µm. From Yetman and Pollack (1986); reprinted by kind permission of Springer-Verlag.
branches, even though this hair is situated approximately midway along the anterior-posterior axis of the proboscis. It would be interesting to learn whether Drosophila is able to aim its proboscis in the direction of the stimulated hair or whether it simply extends the proboscis straight ahead, regardless of the position of the hair that evokes the response. Only one of the seven Drosophila morphological types is strongly reminiscent of a cell type seen in Phormia; this is the type VI cell, which resembles the DBPC of Phormia. Both type VI and DBPC arborize more dorsally than the other gustatory neurons. In both cases, the branches arise at an approximate right angle from the axon, and invade both the ipsilateral and contralateral hemiganglia.

**Tarsal Hairs.** Murphey et al. (1989) stained tarsal hairs of both Phormia and Drosophila with CoCl₂ and found similar results for the two species. The hairs project to the ventral leg neuropil, with the cells from each hair falling into at least three morphological classes (Fig. 4). Four axons arborize ventromedially in their neuromere of origin, and one of these (or, in some hairs, more than one) has an axon that ascends to the head, thus constituting a distinct type (see below). The fifth axon is larger in diameter, has coarser processes, and branches more dorsally and laterally in its neuropile, thus constituting a distinct type (see below). The same region of the subesophageal ganglion as the DBPC. Edgecomb and Pollack (see Edgecomb, 1986) attempted to determine the identity of this receptor using the physiological technique of signal averaging. They recorded simultaneously from the tip of a tarsal hair and from the cervical connective and searched, in averaged recordings, for spikes in the neck connective that followed spikes recorded at the tip of the hair with constant latency; triggering data acquisition with each occurrence of a spike at the hair tip and averaging many such sweeps of data accentuate spikes that are time-locked to the triggering signal. They failed to detect spikes of the mechanoreceptor or of the sugar, classical salt, or fifth cells in the cervical connective (although the same technique was able to extract chemoreceptor spikes from whole-nerve recordings of the maxillary-labelar nerve (Edgecomb, 1986) or of the leg nerve (Murphey et al., 1989)). This leaves the water receptor as the most likely candidate. Unfortunately, they were unable to record water-receptor spikes from tarsal hairs (see above), and so they could not test this possibility directly.

**Genetic Determinants of Axon Projections.** Experiments on Drosophilia have begun to examine the genetic determination of the central projections of taste hairs. Possidente and Murphey (1989) found that chemoreceptor neurons from prothoracic tarsal taste hairs of males often send processes not only to the ipsilateral neuromere but also contralaterally, while in females terminations are strictly ipsilateral (tactile neurons, both from singly-innervated, purely tactile hairs and from taste hairs, terminate only in the ipsilateral hemiganglion, both in males and females). Experiments with genetically mosaic, and phenotypically gynandromorphic flies, in which male legs sometimes project to female central targets, and female legs to male targets, showed that the gender-type of the projection pattern (ipsilateral or bilateral) was strictly correlated with the gender of the leg. This finding indicates that it is the sensory neuron, rather than the central nervous system, that determines the projection type.

Possidente and Murphey (1989) investigated the genetic basis for this sexual dimorphism in axon projections by studying flies that carried sex-transforming mutations. Mutations in the genes tra, tra-2, and Sxl lead to masculinization of external features and of behavior in flies that are chromosomally female (i.e., XX). These masculinized females have male-specific cuticular features including sex combs (specialized tufts of hairs found on the prothoracic tarsi of males) and higher numbers of taste hairs on the tarsi. In two of the mutants (tra and Sxl), gustatory neurons of mutant females sometimes crossed the midline, as they often do in wild-type males, although with lower frequency than is seen for the latter.

In the third sex transforming mutant that was investigated, tra-2, bilateral projections from taste hairs were never observed in females, even though these flies appeared to be fully masculinized. Thus, it seems possible that the gender-specific phenotype of gustatory neurons might differ from the gender of the cuticle (an interesting possibility, given the developmental relationships between cuticular and neuronal components of taste hairs; see below). However, given the small sample size (12) and the relatively low probability of detecting “male-like” axon projections in tra and Sxl...
females (3/12 and 3/13, respectively), the possibility that bilaterally projecting neurons were missed simply by chance cannot be excluded.

A number of investigations in the past few years have elucidated the sequence of genetically controlled decisions that mediate the development of a multicellular sensory organ (reviewed in Ghysen and Dambly-Chaudière, 1993; Ghysen et al., 1993; Jiménez and Modolell, 1993). One of these decisions, controlled by the gene \( \text{poxn} \), determines whether an external sensillum will develop as a singly innervated, mechanosensory organ, or as a multiply innervated, chemosensory organ (Dambly-Chaudière et al., 1992; Nottebohm et al., 1992). This gene, which is expressed in the epidermal precursor cells of multiply innervated sensilla, results in conversion of multiply innervated sensilla into singly innervated ones when it is deleted, and in supernumerary multiply innervated sensilla when it is expressed ectopically. Some of these supernumerary sensilla occur at the same characteristic positions as sensilla that are singly innervated and mechanoreceptive in wild-type individuals; thus, expression of \( \text{poxn} \) in the precursors of these sensilla transforms them from mechanosensory to chemosensory sensillum types.

On the tarsi, singly innervated, mechanosensory hairs and polyinnervated, gustatory hairs can be distinguished by their external morphology; mechanosensory hairs are straight, have sharp tips, and have brachts (cuticular protrusions) at their bases, while chemosensory hairs, both normal and when induced by ectopic expression of \( \text{poxn} \), are curved, have blunt tips, and are brachtless. Nottebohm et al. (1992) studied the central projections and function of tarsal hairs that occupied positions of identified mechanosensory hairs but whose external morphology indicated that they had been transformed, by ectopic expression of \( \text{poxn} \), into chemosensory hairs. Cobalt fills of these transformed hairs revealed groups of fibers that branched within the central region of the leg neuropil, similar to the branching pattern of the chemoreceptors of normal taste hairs. Moreover, stimulation of transformed hairs with water elicited proboscis extension in thirsty flies, suggesting that these hairs contain at least one of the normal complement of chemoreceptor cells, i.e., the water cell, and that this cell is able to make central connections appropriate to its peripheral physiology.

The transformed hairs are not entirely normal taste hairs because they appear (based on their central...
projections) to lack a mechanoreceptor. It will be interesting to learn, from electrophysiological studies, to what extent the physiological characteristics of the neurons associated with transformed hairs mirror those of normal taste hairs.

DEVELOPMENT OF TASTE SENSILLA

General Description

Taste sensilla, like most sensilla of insects (but not all: see Hartenstein and Posakony, 1989; Huang et al., 1991; Technau and Campos-Ortega, 1986), constitute multicellular clones derived from single epidermal precursor cells, the sensory mother cells, or SMCs (reviewed in Bate, 1978). Expression patterns of poxn (Dambly-Chaudière et al., 1992), or of lacZ in Droso phila transformants, in which expression of this reporter gene is under the control of a sensillum-specific promoter (Huang et al., 1991; Ray et al., 1993), demonstrate that these precursor cells have already been parsed from their neighbors in the late third instar larva. During the first 20–30% of the pupal period (Lakes and Pollack, 1990a,b) the SMCs undergo a series of mitoses which produce both the neuronal and (most, at least) nonneuronal components of the sensillum. Once the component cells have been generated, hair formation begins with the outgrowth of dendrites beyond the surface of the epithelium. These are soon followed and enveloped by the elongating process of the thecogen cell (De Kramer and van der Molen, 1984; Hansen and Hansen-Dekeskamp, 1983). Axogenesis begins shortly after the onset of dendritic elongation. The axons of a single sensillum generally travel in a single bundle, with one or more growth cones sending numerous filopodia from the advancing tip of the bundle. Axons from more distal sensilla join with those of their proximal neighbors as they grow towards the main nerve pathways in the developing appendage (Lakes and Pollack, 1990a).

The process of hair formation, from the first protrusion of dendrites beyond the surface of the epithelium until the beginning of cuticle deposition (and thus fixation of hair length), takes some 4–5 days in Proto-
phormia terraenovae at 19° (corresponding to roughly 20–25% of the pupal period; Hansen and Hansen-Delkeskamp, 1983). Throughout much of this period, the tips of the dendrites extend beyond the support cells into the fluid surrounding the developing appendage (wing, leg, or labellum). Interestingly, the lengths of the dendrites of a single developing hair often differ (Hansen and Hansen-Delkeskamp, 1983), suggesting that the different neurons might begin to extend dendrites at different times.

As in other sensory systems (Hartenstein, 1988; Hartenstein and Posakony, 1989; Huang et al., 1991; Lakes and Pollack, 1990a,b; Murray et al., 1984), the generation of different groups of taste sensilla is spatially and temporally organized (Fig. 7). For example, labellar taste hairs of Drosophila arise in three waves, with the earliest hairs appearing medially and midway along the length of the labellum, and later hairs arising more laterally, anteriorly, and posteriorly (Ray et al., 1993). In Phormia, labellar hairs appear to arise in a more loosely organized fashion, with new hairs appearing interspersed among older ones. The taste papillae, however, arise according to a clear spatial and temporal pattern which originates midway along the length of the lateral edge of the labellum and radiates medially, anteriorly, and posteriorly (Pollack and Lakes-Harlan, 1995).

Lineages of Sensilla

Two important questions concerning the development of taste sensilla are: 1) are the component cells of a sensillum generated by a strict, stereotyped, lineage and, if so, what is its structure, and 2) how are the phenotypes of the cells determined? These are of course related questions, because a strict lineage pattern, if it exists, might play a direct role in determining cellular phenotypes.

The first division of the SMC produces two second-generation precursor cells, one of which undergoes a second, and terminal, mitosis to produce the trichogen and tormogen cells (Peters, 1965), while the other has a more complicated destiny (see below). In mechanoreceptive hairs of Drosophila the different fates of these two second-generation precursors are determined by the gene numb, the protein product of which is asymmetrically distributed in the SMC and thus unequally delivered to its daughters (Rhyu et al., 1994). The phenotypes of numb mutants and of flies in which numb is expressed ectopically indicate that the Numb-deficient daughter of the SMC divides to produce the trichogen and tormogen cells, and that its Numb-rich sister generates the remaining cells of the sensillum. Overexpression of numb also influences cell fate at later stages of sensillum development, indicating that its role may not be restricted to the first division of the SMC.

In mechanoreceptive hairs, one of the SMC daughters (presumably the Numb-rich one) divides to produce the single neuron of this sensillum and thecogen cell (Hartenstein and Posakony, 1989; Rhyu et al., 1994). Interestingly, induced expression of poxn can alter the fate of presumptive mechanosensilla even after both SMC daughters have completed what would have been their terminal mitoses. Although such late expression of poxn fails to alter the external appearance of the sensilla, it does result in the generation of clusters of up to five neurons. These are found near, but do not actually connect with, the hairs, which are thus left noninnervated. This unusual arrangement probably arises from the mismatch between hair and neuron types; the hair is mechanosensory, and lacks the lumen in which dendrites are found in chemosensory hairs. At
the same time, the chemosensory neurons lack the cellular specializations that allow mechanoreceptors to attach at the base of the hair (Nottebohm et al., 1994).

The structure of the lineage that generates the group of neurons and the thecogen cell of chemosensory hairs has been worked out by determining the age relationships of the various cells of a sensillum. Developing pupae are injected at various stages with the thymidine analog 5-bromo-2’-deoxyuridine (BrdU), which is incorporated into DNA that is synthesized during the relatively brief period during which BrdU remains available for uptake (Hartenstein and Posakony, 1989; Pollack and Lakes-Harlan, 1995). After development is completed, the distribution of BrdU-labeled cells in a sensillum is assayed by anti-BrdU immunocytochemistry. By determining when various cells of the sensillum undergo their last round of DNA synthesis in preparation for their final mitosis, it is possible to infer which cells are, and which are not, siblings (e.g., a cell which has incorporated BrdU cannot be the sibling of a cell which has not, whereas two cells are likely to be siblings if, e.g., they are the only ones in the sensillum to have incorporated BrdU following injection at a particular stage; Hartenstein and Posakony, 1989).

**Taste Hairs**

Ray et al. (1993) injected Drosophila pupae with BrdU at defined stages in the development of identified labellar taste hairs. In addition, they counted the number of cells in a developing sensillum at various stages, both by using an antibody that stains these cells in Drosophila, and by using a transformed Drosophila line in which beta-galactosidase is expressed in the SMC and all of its progeny (Huang et al., 1991). Injections made early within the hairs’ lineages, when the developing cluster contained only two cells (the SMC daughters), most often labeled all the cells of the sensillum; the BrdU was incorporated by the SMC daughters and inherited by their progeny. In a few cases, however, only the trichogen and tormogen cells were labeled. This result indicates 1) that the trichogen and tormogen cells are siblings, and 2) that the mitoses of the two SMC daughters are not strictly synchronous. The occasional failure of the neurons and thecogen cell to incorporate BrdU comes about because, in these cases, the by the “neurogenic” SMC daughter began DNA synthesis, BrdU was no longer available. This interpretation is validated because BrdU injections later in the two-cell stage of the sensillum consistently overlap the 5 phases of both SMC daughters, and thus label all of the neurons as well as the trichogen, tormogen, and thecogen cells. Still later injections, when the developing sensillum has four cells, label only 4 of the 5 neurons. This labeling pattern can be accounted for as follows. One of the SMC daughters has already divided to produce the trichogen and tormogen cells, thus accounting for 2 of the 4 cells in the developing sensillum. A third cell is a granddaughter of the SMC, which will divide again to produce the thecogen cell and 1 of the 5 neurons, and the fourth is another SMC granddaughter, which will undergo two rounds of mitosis to produce the remaining four neurons. The failure to label the thecogen cell and one of the neurons suggests that their precursor has not yet begun DNA synthesis at this time. Its sister, however, has done so.

Injections made slightly later in the four-celled stage label all five neurons and the thecogen cell, presumably because at this time the mother of the thecogen cell and its sister neuron have begun DNA synthesis, but the grandmother of the remaining neurons has not yet completed DNA synthesis. The lineage derived from these experiments is diagramed in Figure 8.

This lineage does not include the glial cell that is normally associated with the sensillum. Moreover, in Drosophila, the dendrites of taste hairs are wrapped by the processes of four accessory cells, rather than three (Falk et al., 1976; Nayak and Singh, 1983). The identity of the fourth cell is unknown, as is its origin.

In Phormia, taste hairs cannot readily be identified as unique individuals during development. Pollack and Lakes-Harlan (1995) therefore studied the distributions of BrdU-labeled neurons per sensillum in populations of developing hairs. Although they injected pupae at a number of developmental ages, new taste hairs arose throughout the time period covered by these injections, and thus each injection captured, in different sensilla, the complete range of developmental stages. Pollack and Lakes-Harlan found that, of the five neurons of the taste hair, either 0, 2, 3, or 5 incorporated BrdU, with a single instance of a hair with 1 of the 5 neurons labeled. In Phormia, a marker for the thecogen cell is not available, so it was not possible to determine whether one of the neurons is the sister of the thecogen cell. The labeling distributions are consistent with the lineage proposed by Ray et al. (1993) with the provisos that the two precursors that generate the two pairs of sibling neurons divide asynchronously, and that one of these precursors divides synchronously with the precursor that generates the thecogen cell and the remaining neuron (Fig. 8).

**Taste Papillae**

Development of taste papillae has been studied only in Phormia (Pollack and Lakes-Harlan, 1995). There are two distinct groups of papillae; a single row of ca. 28 lateral papillae situated at the lateral edge of the oral surface, one at the end of each pseudotrachea, and a group of ca. 40 interpseudotracheal papillae scattered over the remainder of the oral surface (Wilczek, 1967). The lateral papillae generally have three neurons each, and the interpseudotracheal papillae have four neurons (Pollack and Lakes-Harlan, 1995). BrdU injections label either 0, 2, or all 4 of the neurons of interpseudotracheal papillae (Fig. 9), strongly indicating that the four neurons constitute two pairs of siblings. Although the nonneuronal cells cannot easily be visualized in Phormia, a pair of BrdU-labeled nuclei is often seen near the apical portion of the neuronal cluster (Fig. 9); these are good candidates for the nuclei of the trichogen and tormogen cells. Either both or neither of these nuclei are labeled in a given sensillum, indicating that they belong to sister cells. There is a tendency for these nuclei to label more frequently in recently-born sensilla (which can be identified based on the spatiotemporal pattern of papilla differentiation). Thus, it is likely that, as in taste hairs, the trichogen and tormogen cells are siblings, and appear early in the lineage of the sensillum. An additional BrdU-labeled nucleus is often found adjacent to the neuronal nuclei (Fig. 9), and is a candidate for the nucleus of the thecogen cell (though it
must be pointed out that the number of accessory cells associated with taste papillae, and thus the existence of a thecogen cell, is unclear; see Pollack and Lakes-Harlan (1995) for further discussion). If this is the thecogen cell's nucleus, then taste papillae differ from taste hairs (as well as from other external sensilla of flies; Bodmer et al., 1989) in that the thecogen cell is not the sibling of a neuron. This conclusion derives both from the labeling distributions, which indicate that the four neurons comprise two sets of siblings, and from the frequent observation of papillae in which all four neurons are labeled with BrdU, but with no other BrdU-labeled nuclei in the immediate vicinity.

If it is assumed that the nonneuronal nuclei that are found associated with papillae belong to the trichogen, tormogen, and thecogen cells, then it seems possible that the lineage of the interpseudotracheal papilla is an "edited" version of that for a hair, with the neuron count reduced from 5 to 4 by elimination (perhaps through programmed cell death?) of the thecogen cell's sibling. Alternatively, the precursor that, in hairs, divides to form the thecogen cell and its sister might, in papillae, differentiate directly into the thecogen cell (Fig. 8).

Although we now know that taste sensilla do arise according to stereotyped lineages, we still do not know whether particular neurons occupy fixed positions within the lineage, e.g., whether the sister of the thecogen cell is always the sugar receptor neuron, or the water receptor, etc., or whether, among the two sets of siblings that comprise the remaining four neurons, particular neurons are always siblings. If a strict relationship between position in the lineage and phenotype does exist, then determination of phenotype by lineage-dependent mechanisms, such as unequal partitioning of substances between daughter cells (similar to the way in which Numb acts), is possible. If, on the other hand, phenotype and lineage position are not strictly related, then other mechanisms for determination of cell fate, e.g., cell-cell interactions, would be necessary.

The reason the relationship between lineage and phenotype is still unknown is that, until recently, no morphological markers for the various neurons existed (save for the presence of a tubular body in the mechanoreceptor, which can be assayed only through the laborious process of electron microscopic reconstruction). Recently, however, Buchner et al. (1993) found that mechanoreceptor neurons of Drosophila, probably including those associated with taste hairs, are histamine-immunoreactive. Thus, it should now be possible, by combining BrdU labeling with histamine immunocytochemistry, to determine whether the mechanoreceptor occupies a consistent position within the lineage of a taste hair. It should be possible to ask this question of other receptor neurons as well, once cell-specific markers become available. Because both biochemical (Ozaki et al., 1993) and genetic (Rodrigues and Siddiqi, 1981; Siddiqi et al., 1989) approaches are coming closer to
isolating and identifying at least some of the receptor molecules, it can be expected that probes for these, in the form of antibodies and/or nucleic acids, will be available before too long.

**NOTE ADDED IN PROOF**

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