Peripheral and Central Structures Involved in Insect Gustation

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ABSTRACT   Studies in insect gustation have a long history in general physiology, particularly with work on fly labellar and tarsal sensilla and in the general field of insect-plant interactions, where work on immature Lepidoptera and chrysomelid beetles has been prominent. Much more emphasis has been placed on the physiological characteristics of the sensory cells than on the central cellular mechanisms of taste processing. This is due to the fairly direct access for physiological experimentation presented by many taste sensilla and to the obvious importance of tastants in insect feeding and oviposition behaviour. In some of the insect models used for gustatory studies, advances have been made in understanding the basic morphology of the central neuropils involved in the first stages of taste processing. There is much less known about the physiology of interneurons involved. In this review, we concentrate on four insect *models (Manduca sexta*, *Drosophila melanogaster*, *Neobellieria bullata* (and other large flies), and *Apis mellifera*) to summarize morphological knowledge of peripheral and central aspects of insect gustation. Our views of current interpretations of available data are discussed and some important areas for future research are highlighted. *Microsc. Res. Tech. 47:401–415, 1999.*   © 1999 Wiley-Liss, Inc.

INTRODUCTION

In the past few decades, much progress has been made on many elements of the chemical senses in insects. In this decade, reviews on the insect taste system have focused on morphology of the sensory structures involved in reception and their development (Keil, 1997; Zacharuk and Shields, 1991); pharmacology, transduction, and structure activity (Mullin et al., 1994); coding of chemical information and intracellular mechanisms (Frazier, 1991), and the organization of afferent terminals in the central nervous system (CNS) (Pollack and Balakrishnan, 1997). Our goal in this paper is to review a selected portion of the insect gustation literature, emphasizing structural elements from the sensilla that transduce chemical information to the structure and organization of the gustatory regions of the CNS, concentrating on those species about which we have the most information for both peripheral and central structures, namely the larval Lepidoptera, especially the hawkmoth *Manduca sexta*; the honeybee *Apis mellifera*; the fruitfly *Drosophila melanogaster*; and the larger blowflies and fleshflies of the families Calliphoridae (*Phormia* and *Calliphora* spp.) and Sarcophagidae (*Neobellieria* [= *Sarcophaga*] bullata).

STRUCTURE OF CONTACT CHEMOSENSILLA

The typical insect taste sensillum consists of a specialization of the cuticle innervated by the dendrites of one or more bipolar sensory nerve cells supported and ensheathed by 3–4 accessory cells at the base (Zacharuk and Shields, 1991; Fig. 1a,d). The classification of sensillum types in insects is done on the basis of cuticular morphology supported by studies of ultrastructure and electrophysiology. Schneider's (1964) classification of insect sensilla into 10 morphological types is still routinely used, modified by the realization that there may also be functional differences among sensilla in a single morphological category, and that novel sensilla also exist that have since been described (Zacharuk, 1985). Within each of the 10 morphological types described by Schneider (1964), there are variations in the number of small pores on the cuticle that are believed to be due to functional differences. The pore numbers vary from aporous, considered to be mechanosensory, to uniporous, considered to be gustatory though often with possible mechanosensory innervation, to multiporous, which are considered to be olfactory (Schneider, 1964; Zacharuk, 1985; Zacharuk and Shields, 1991). Of these sensilla types, the ones believed to mediate gustation are uniporous. The 4 types commonly thought to have a contact chemosensory function (Zacharuk and Shields, 1991) are:

1. *Sensilla trichodea* are freely moveable setiform hairs of variable lengths, with their bases set in the basal membrane in a variety of different insertion types. Their diameters are generally proportional to their lengths, and they have been ascribed as having mechanosensory function with some also having chemosensory and/or thermosensitive functions.
2. *Sensilla chaetica* are similar to sensilla trichodea except that these bristles and hairs are set in a socket and can be identified by their thick walls. They are usually considered to have tactile and sometimes chemosensory function.

1a

1b

0.25 µm

1c

0.2 µm
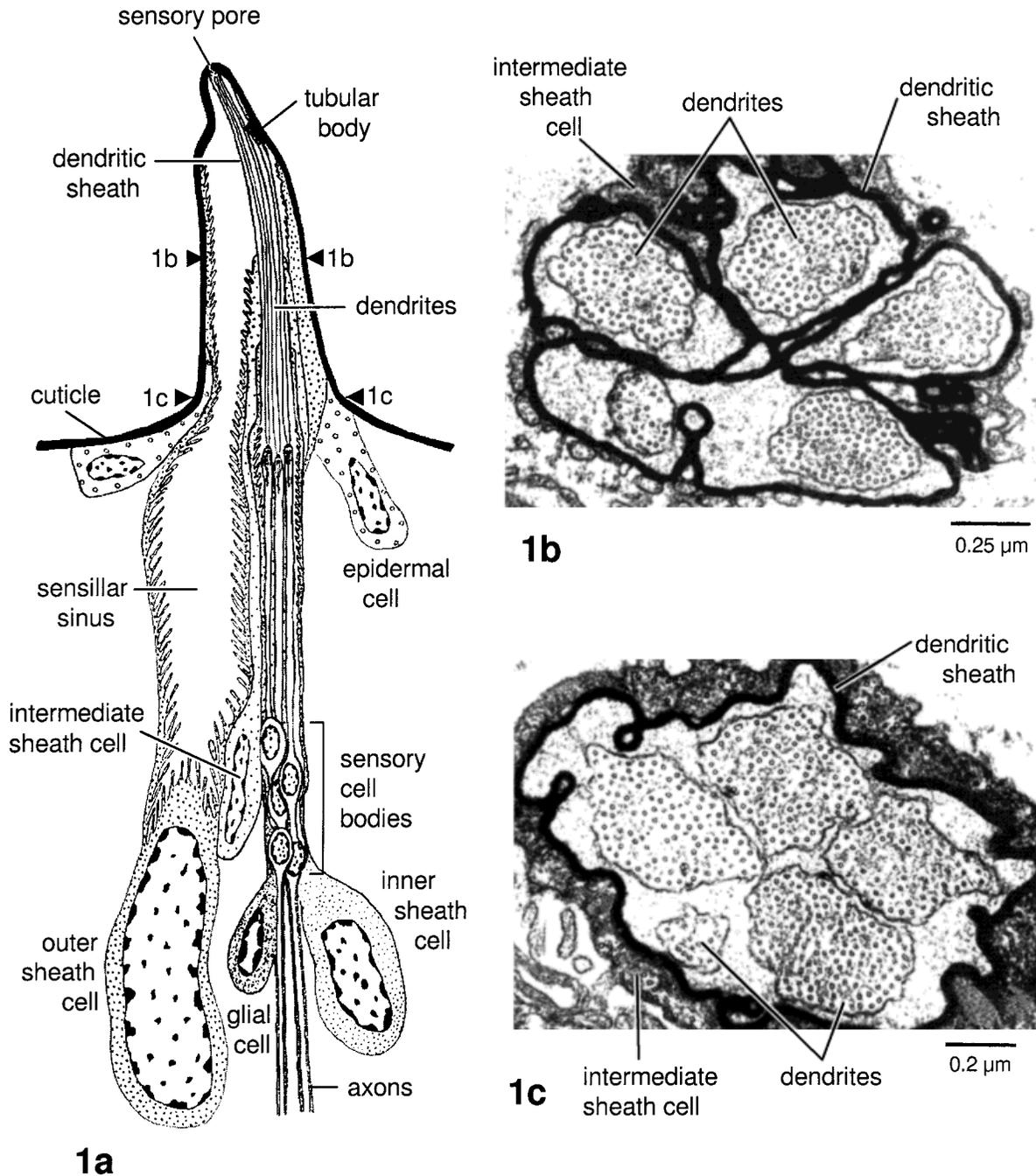
Fig. 1. a–c: Diagramatic reconstruction and ultrastructural aspects of a typical larval lepidopteran uniporous sensillum styloconicum present on the maxilla. **a:** Reconstruction of styloconic sensillum of *Mamastra configurata* from EM sections. Features common to this type of sensillum in all Lepidoptera studied are labeled. Arrowheads refer to levels of sections in b and c. **b:** Cross-section of a styloconic sensillum approximately at the level indicated in a (arrowheads). One of these dendrites innervates the base of the sensillum at the top of the style and is mechanosensory, the other four are chemosensory. The dendritic sheath is elaborate at this level, surrounding individual dendrites and presumably providing support for them. **c:** Proximal to b, the dendritic sheath is less elaborate and the five dendrites mingle in the same space. One of the surrounding sheath cells responsible for secreting the sheath is clearly seen. This section is approximately at the level indicated in a. a–c modified from Shields (1994). d–g: Diagramatic reconstruction and ultrastructural aspects of a typical adult dipteran taste hair. **d:** Diagram of a labellar taste hair in *D. melanogaster*. Features common to this type of sensillum in all flies studied are labeled. The two-chambered nature of such sensilla is apparent, with the smaller chamber providing support for the dendrites. Here, the mechanosensitive cell terminates at the base of the hair in a distinct tubular body. **e:** The tips of these sensilla are difficult to study in TEM and relatively few photographs have been published. This longitudinal section through a labellar sensillum of *P. regina* clearly shows the two lumina with the one containing the four chemosensitive dendrites in communication with the environment through the single apical pore. **f:** The cross-sections of *P. regina* sensilla (Fig. 1f,g) can be compared with those shown to represent Lepidoptera (b and c). In mid-shaft (**g**), the four chemosensory dendrites are clearly seen as are the two lumina. f is proximal to g and proximal to the tubular body of the mechanosensory cell (d). The fifth dendrite is the mechanosensitive cell and it is completely surrounded by the dendritic sheath. d modified from Nayak and Singh (1983); e modified from Sturckow et al. (1973); f,g modified from Felt and Vande Berg (1976). Scale bar in e estimated from published print.

1d

sensillar sinus

tubular body

dendritic sheath

outer sheath cell

sensory cells

axons to subesophageal ganglion

1e

terminal pore

0.5 µm

dendritic channel

1f

mechanoreceptor

dendritic sheath

0.5 µm

1g

0.5 µm

dendritic sheath

sensillar sinus
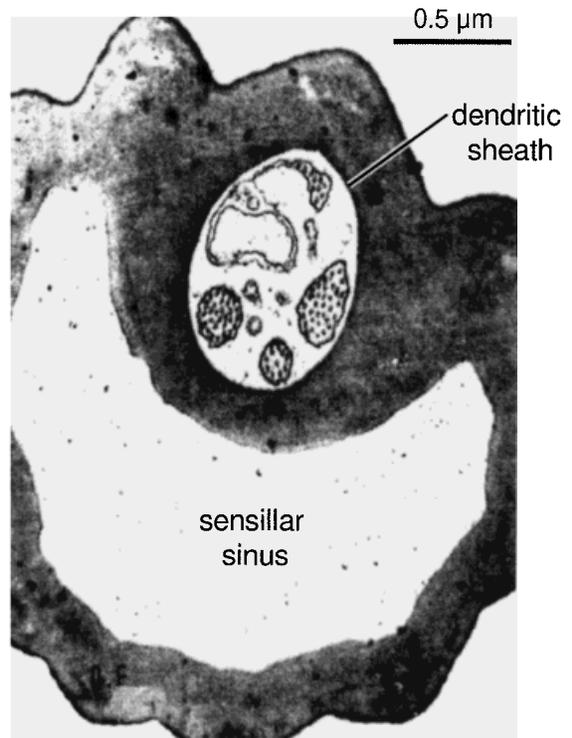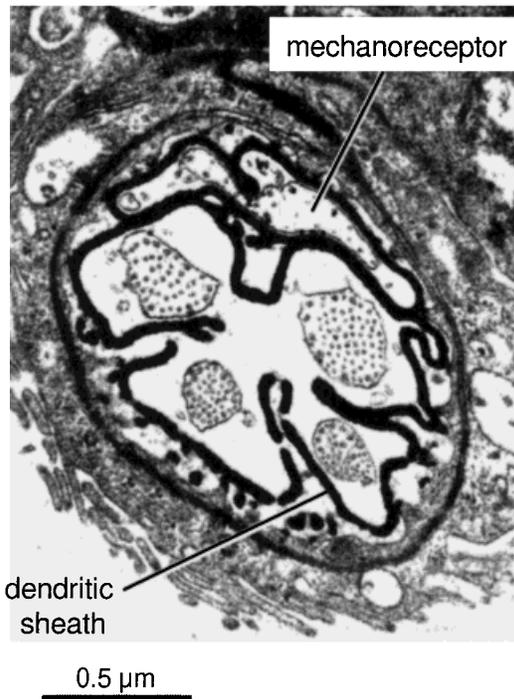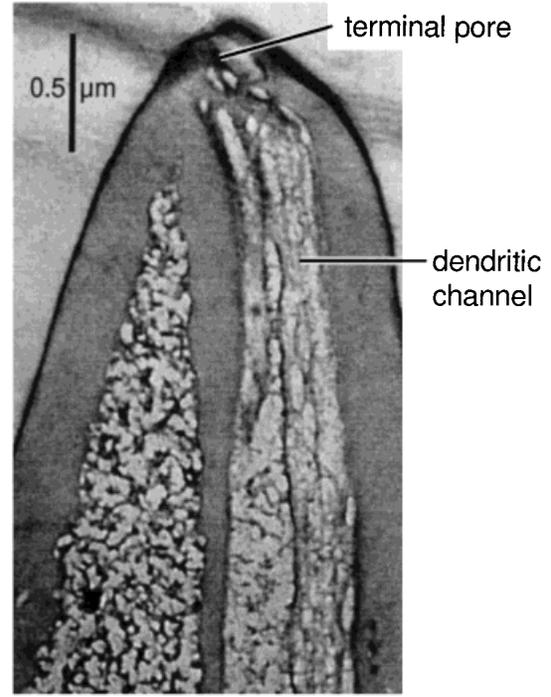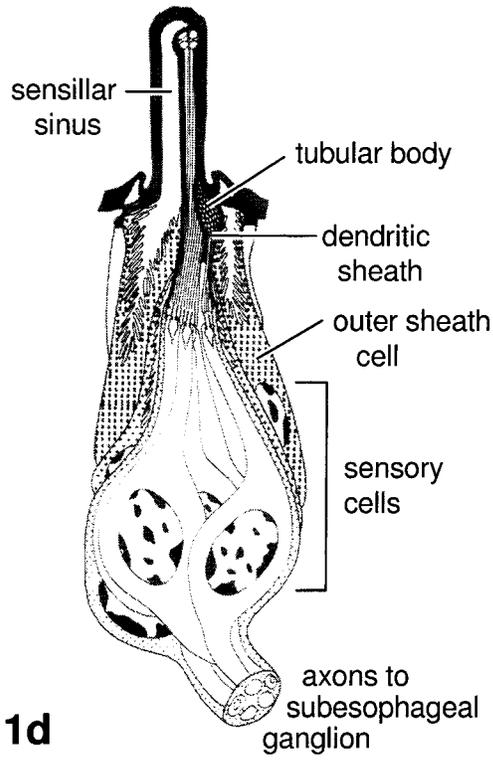
Fig. 1.   (Continued.)

3. *Sensilla styloconica* are peg-like, set on top of a conical cuticular style, and have been shown to have mechanosensory and/or chemosensory functions.
4. *Sensilla placodea* are cuticular plates generally set at the level of the cuticle, attached to it by a ring of membrane.

Much of what we know about gustatory sensilla has come from work on the mouth parts of insects due to the central role these sensilla play in feeding behavior. However, investigators have also recognized that gustatory receptors on the ovipositor play a role in host recognition for oviposition (e.g., Anderson and Hallberg, 1990), while other receptors on the tarsi are potentially used in both feeding and oviposition (Anderson and Hallberg, 1995; Baur et al., 1998; Hansen and Heumann, 1971; van der Wolk, 1978; van der Wolk et al.,1984).

In general, the uniporous sensilla with putative gustatory function have similar morphologies at the ultrastructural level. All have a single pore, either at or near the tip of pegs or in the middle of plates. These pores may be simple or stellate in shape, and they may be elaborated with grooves converging at the pore or more complex finger-like projections that surround the opening (Zacharuk, 1985). Internally, the dendrites of the sensory neurons typically project to near the pore, surrounded by a dendritic sheath that divides the sensilla lumen into two compartments: the dendritic chamber and the sensillar chamber. The dendritic chamber is typically innervated by 4–6 sensory neurons, though in various insects, as few as one and as many as 10 have been described (Zacharuk, 1985). Also, some of the dendrites appear to branch within the sheath. In most uniporous sensilla, which have both a mechanosensory and gustatory function, a single sensory cell is separated from the others by a septum within the dendriitic sheath below the base of the sensillum. This cell is mechanosensitive and terminates in a tubular body at the base of the sensillum (Zacharuk, 1985).

Here, we concentrate on the morphology of gustatory sensilla in four groups of insects about which we have the greatest amount of information on both the peripheral and central structures involved in the processing of gustatory information.

LARVAL LEPIDOPTERA, ESPECIALLY THE HAWKMOTH *MANDUCA SEXTA* (SPHINGIDAE: LEPIDOPTERA)

The gustatory sensilla of larval Lepidoptera have received intense scrutiny due to their importance as agricultural pests. Behavioral studies have shown that olfaction is used in the initial orientation and discrimination of possible foods, while gustation is used for the final discrimination that initiates feeding (DeBoer and Hanson, 1987; Hanson and Dethier, 1973). Hence, the gustatory sensilla can be regarded as the ultimate arbiters of what plants are ingested or rejected.

Among Lepidoptera, larvae of the hawkmoth, *M. sexta*, have become a model system for the study of insect gustation, especially with respect to insect-plant relationships (e.g., DeBoer, 1991a,b, 1992; DeBoer and Hanson, 1987; Dethier and Crnjar, 1982; Dethier and Kuch, 1971; Dethier and Schoonhoven, 1969; Hanson

and Peterson, 1990; Peterson et al., 1993; Schoonhoven and Dethier, 1966). In general, the elongate *sensilla styloconica* of the maxilla are the main larval gustatory organs (Dethier and Kuch, 1971; Kent and Hildebrand, 1987; Schoonhoven and Dethier, 1966) (Fig. 1). Each maxilla has 2 *sensilla styloconica* on the galea, one located laterally and one medially, each of which has four gustatory receptors (Schoonhoven and Dethier, 1966). Each maxillary palp has 8 *sensilla basiconica* at its tip, with a total of 14–19 receptor neurons (Schoonhoven and Dethier, 1966). The *sensilla basiconica* on the palp are of 2 morphological types: 3 with multiple pores, believed to be olfactory sensilla, and 5 with a single pore, believed to be gustatory sensilla (Dethier and Crnjar, 1982; Hanson and Dethier, 1973).

The 4 gustatory cells in the *sensilla styloconica* on the maxillary galea have strikingly different sensitivities, with the first being maximally sensitive to sucrose/glucose; the 2nd to NaCl; the 3rd to inositol; and the 4th to plant alkaloids such as salicin (Dethier and Crnjar, 1982). Other putative gustatory receptors have been identified on the epipharyngeal surface of the labrum, and there is some evidence for other receptors in the hypopharynx and deeper portions of the buccal cavity (Kent and Hildebrand, 1987).

Some of these gustatory sensilla in *M. sexta* and other larval Lepidoptera have been studied in ultrastructural detail by a number of investigators (Devitt and Smith, 1982; Ma, 1972; Schoonhoven and Dethier, 1966; Shields, 1994a,b; Wieczorek, 1976). Of these gustatory receptors, the *sensilla styloconica* on the galea of the maxilla are the best studied (Fig. 1a–c), partly due to the fact that they can be characterized electrophysiologically, so that more detailed structure-function questions can be asked. In *M. sexta* larvae, Schoonhoven and Dethier (1966) found that the medial and lateral styloconic sensilla on the galea of the maxilla were morphologically identical, with the 4–6 dendrites being surrounded in the apical regions of the sensillum by a sheath that separated them from the sensillar chamber. As only four neurons are believed to innervate the tip of the styloconic sensillum, it is probable that one or more of the chemosensory dendrites branched as they near the apical pore.

In other larval Lepidoptera, ultrastructural studies of the *sensilla styloconica* on the maxilla have found very similar results. They all find 4–6 dendrites enclosed in a sheath that separate them from the sensillar chamber, with some dendrites branching on their projection to the apical pore (*Euxoa messoria:* Devitt and Smith, 1982; *Pieris brassicae:* Ma, 1972; *Mamestra configurata:* Shields, 1994a,b). This well-studied system is illustrated using figures from Shield's (1994a,b) work on *M. configurata* (Fig. 1a–c).

Honeybee, *Apis mellifera*

The honeybee has been a favored organism for the investigation of insect communication and learning (von Frisch, 1967), in which chemosensory pathways play a major part. However, the ultrastructure of honeybee contact chemoreceptors is relatively less studied, with Slifer and Sekhon (1961) investigating those found on the antenna, and Whitehead and Larsen (1976a,b) investigating receptors on the head (antenna, mouth parts) and on the foretarsi. In the latter study,

sensilla chaetica and *sensilla basiconica* were identified as the gustatory sensilla on the head and legs of the honeybee.

On the antenna, some 300 gustatory *sensilla chaetica* are present, but no *sensilla basiconica*. On the different regions of the mouth parts (glossa, labial palps, galea), approximately 120 *sensilla chaetica* and 90 *sensilla basiconica* with gustatory function have been counted. On the forelegs, each tarsomere has 10–21 *sensilla chaetica* and 0–6 *sensilla basiconica*, both types are thought to be gustatory (Whitehead and Larsen, 1976a,b). Ultrastructural work on these receptors revealed that their fine morphologies are very similar to that of other uniporous insect sensilla. Both the glossal and maxillary *sensilla chaetica* and *sensilla basiconica* have 4 dendrites in a dendritic sheath that separates them from the sensillar chamber, with a pore visible at the tip of some of the sections. As well, a single mechanosensory neuron is located at the base, and its dendrite is associated with a tubular body (Whitehead and Larsen, 1976a,b).

Fruitfly, *Drosophila melanogaster*

Due to its well-understood genetics, *D. melanogaster* has been a model organism for many studies, including those investigating the organization of chemoreceptors and their projections. The gustatory receptors are widely distributed in the adult *D. melanogaster*, as they have been found on the labellum, the pharynx, the wing margins, the legs, and the female genitalia (Stocker, 1994).

The labellar gustatory receptors in *D. melanogaster* have been classified into two types, taste bristles and taste pegs, which appear to be analogous to the *sensilla trichoidea* and *sensilla basiconica* in larger flies (Falk et al., 1976). Between 36–42 taste bristles are located on each half of the labellum, with most bristles having four chemoreceptor neurons, and a few having only two. The dendrites of these neurons are located in a lumen within the sensillum, distinct from a second lumen that is filled with sensillar lymph. Most labellar taste bristles end with a blunt, two-pronged tip, with a fold between these tips that may hold the sensillar pore, while some single-tip bristles end with a pore. As in other uniporous insect sensilla, a single mechanosensory neuron sends a dendrite to the base of each bristle (Falk et al., 1976). Also on the labellum, taste pegs (*sensilla basiconica*) are found between the folds of the pseudotracheae. Between each pair of pseudotracheae are 4–7 taste pegs, totalling some 30 on each half of the labellum (Stocker, 1994). Each of these sensilla apparently has only one chemosensory cell, as in the distal part of the sensillum near the pore only one dendrite can be seen. As in the taste bristles, each taste peg is innervated by a single mechanosensory cell, and has an internal organization essentially identical to the labellar taste bristles (Falk et al., 1976). The physiological responses of the labellar bristles/hairs show the presence of receptors responding to sucrose, NaCl, and water (Rodrigues and Siddiqi, 1978; Shanbhag and Singh, 1992; Stocker, 1994).

In the *D. melanogaster* pharynx, 5 groups of sensilla have been identified, of which the labral sense organ (LSO) and the ventral and dorsal cibarial sense organs (VCSO, DCSO) have been shown to have chemosensory neurons (Nayak and Singh, 1983; Stocker, 1994) (Fig. 2c). The ultrastructure of a chemoreceptor in the LSO shows the typical morphology of the chemosensory dendrites enclosed within a sheath, though the sensillum pore is at the level of the cuticular surface with the rest of the structure below the cuticle (Nayak and Singh, 1983).

D. melanogaster legs are covered with four types of sensilla, of which the taste bristles, with morphology very similar to those on the labellum, appear to be gustatory. They have a terminal pore and two lumina; four dendrites of gustatory neurons are located in one of these lumina. Again, a mechanosensory cell innervates the base of the sensillum (Nayak and Singh, 1983; Stocker, 1994). About 30 bristles are typically located on each leg on the tibia and tarsi, with forelegs having a few more than the meso- and metathoracic legs, and male forelegs having about a third more bristles than those of females (Nayak and Singh, 1983; Shanbhag and Singh, 1992). These taste bristles are responsive to salt and sugar (Hannaford and Palka, 1992).

On the wings, some 30 chemosensitive taste bristles much like those on the labellum are located on the dorsal surface along the costal veins, with an additional 12 on the ventral surface. These bristles display a terminal pore, with four dendrites in their shaft enclosed in a thin lamella, and a mechanosensory dendrite at the base of the bristle (Palka et al., 1979). Behavioral assays indicate that these wing taste bristles are responsive to both sugar and salt (Hannaford and Palka, 1992; Stocker, 1994).

Based on morphological criteria, 6 *sensilla trichodea* on the vaginal plate of female *D. melanogaster* have been identified as having chemosensory function. As well, an additional 10–15 shorter bristles also on the vaginal plate appear to have the morphology of chemosensory hairs (Stocker, 1994).

Flies of the Families Calliphoridae and Sarcophagidae

The gustatory sensilla in blowflies and fleshflies have been well studied, due in great part to the ease with which the responses of the contact chemoreceptive cells can be recorded in vivo (e.g., Dethier, 1976; Wieczorek et al., 1988). As in the smaller *D. melanogaster*, the gustatory receptors in these flies have been localized to the labellum, the tarsi, and the female genitalia (Stocker, 1994). On the labellum, although the number of taste receptors is much higher than in *D. melanogaster*, the general organizational plan is maintained in the blowflies and fleshflies. The taste bristles (*sensilla trichodea*) are substantially longer (15–350 µm in length) than the taste pegs between the pseudotracheae of the labellum (6–10 µm) (Van der Wolk et al., 1984). In *P. regina*, the taste bristles on each labellum number some 125, while between the pseudotracheae, the papillae number about 65 (Wilczek, 1967). In comparison, the labellar taste bristles number about 130 per labellum in *Calliphora vicina* (Maes and Vedder, 1978) while in *C. erythrocephala* they number 128 (Wilczek, 1967). Despite the differences in sensillar number, the taste bristles are structurally and functionally the same as in *D. melanogaster*, having two chambers within the shaft, with the dendrites of the chemoreceptors separated from the sensillar lymph (Dethier, 1976; Stocker,

2a

2b

2c

2d
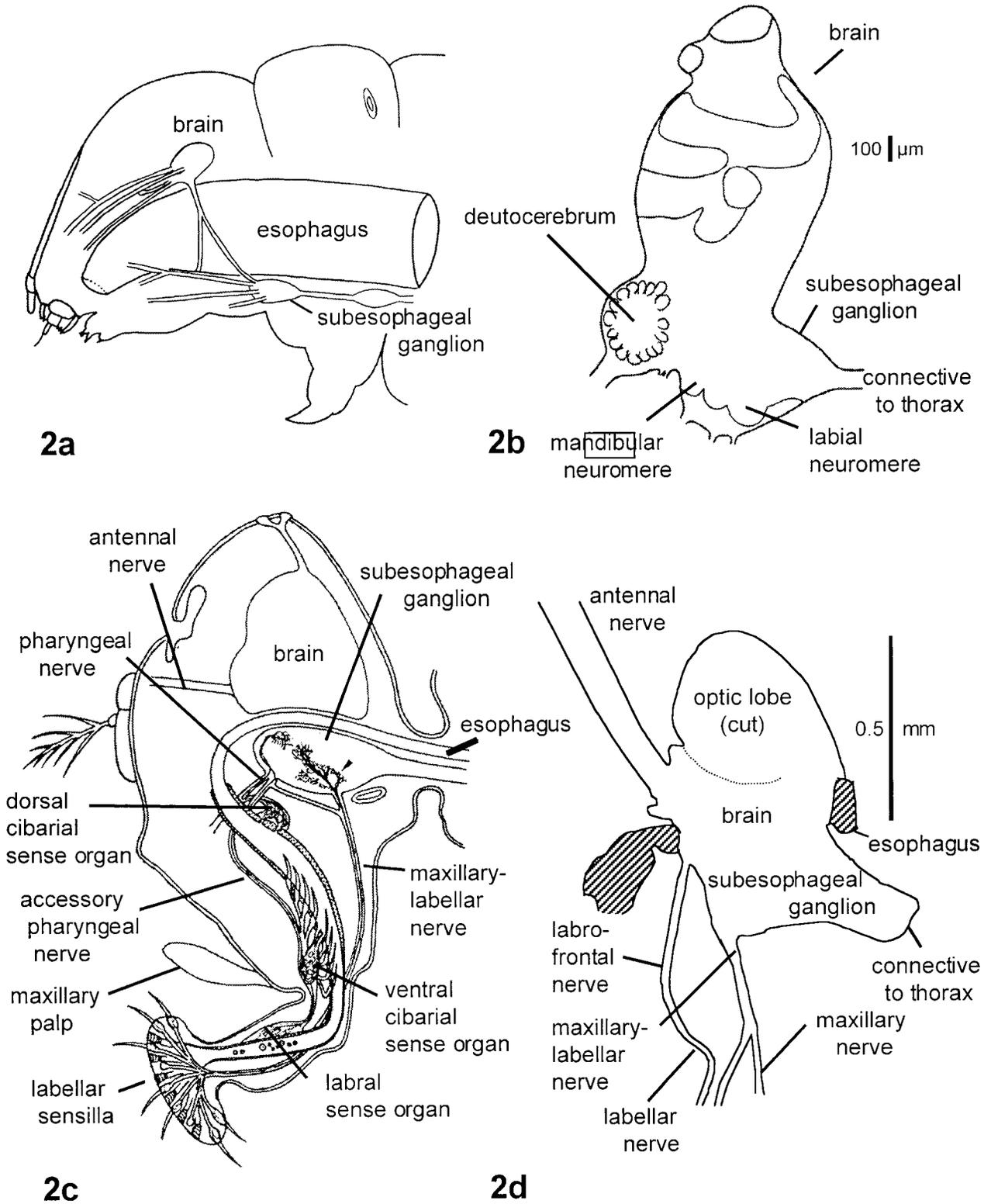
Fig. 2. The SOG bears various relationships to the brain in the insects highlighted in this review. In larval lepidopterans (**a**), it is largely separated from the brain, connected only by relatively long circumoesophageal connectives. In the honeybee (**b**), there is significant fusion between the brain and the SOG and the three mouth part neuromeres (maxillary, mandibular, and labial) are discernable in saggital sections. In the flies (**c,d**), brain and SOG are also fused.

However, the lack of a mandibular neuromere and strong fusion of the maxillary and labial neuromeres make it difficult to separate these at the level of gross morphology. a,b, and c are modified, respectively, from Kent and Hildebrand (1987) (*M. sexta*), Rehder (1989) (*A. mellifera*), and Stocker and Schorderet (1981) (*D. melanogaster*). d was drawn from a photograph of a dissection of a *N. bullata* brain.

1994) (Fig. 1e,g). In these larger flies, four dendrites are typically found within the bristles, although 3 and 5 dendrite profiles are occasionally seen, along with the single mechanosensory neuron (Fig. 1d,f) (Dethier, 1976; Larsen, 1962). These chemoreceptive cells respond to sugars, salts, and water (Dethier, 1976; Maes and Vedder, 1978; Shiraishi and Morita, 1974). More recently, investigators have delved into the development of these labellar taste hairs (De Kramer and Van Der Molen, 1984; Pollack and Balakrishnan, 1997).

The tarsal taste receptors have also received substantial study, with the majority of them located on the ventral and ventrolateral aspects of the tarsi, and a few located on the dorsal aspect of the tibia. In *P. regina*, they number some 300 on the prothoracic leg, 200 on the mesothoracic leg, and 150 on the metathoracic leg (Dethier, 1976; Grabowski and Dethier, 1954). Similar sensilla numbers were seen on the prothoracic tarsi in *P. terranovae*, while in *C. vicina*, they number some 190, and in *Musca domestica* about 370 (Van der Wolk, 1978). The ultrastructure of these tarsal sensilla are essentially the same as those on the labellum (Stocker, 1994). In the less closely-related phytophagous Anthomyiid fly *Delia radicum*, the tarsal taste sensilla have the archetypical two chambers with the four chemosensory dendrites and the mechanosensory cell at the base (Isidoro et al., 1994).

Salt sensitivity of the wings was first described in *P. regina* (Wolbarsht and Dethier, 1958) and it is likely that the sensilla transducing this response are similar in structure to other taste bristles found on these flies. As for the female genitalia, this has been investigated only in the blowfly *Lucilla cuprina*, where the cerci have ten trichoid sensilla, which have their dendrites enclosed in a sheath as in other known gustatory sensilla. Interestingly, some of these sensilla have 3 dendrites in the sheath without a mechanosensory cell at the base, while others have the standard 4 dendrites with an additional mechanosensory cell (Merritt, 1987; Merritt and Rice, 1984). These cercal sensilla are known to be salt-sensitive (Rice, 1977).

NEUROANATOMY OF TASTE PROCESSING IN THE SUBOESOPHAGEAL GANGLION

Taste cells have been described from numerous parts of the insect body, yet, as with many sensory modalities, structures on the head provide the majority of gustatory information. The first stage of processing of gustatory input often occurs in the same segment as the sensilla being stimulated. However, the SOG, in addition to receiving input from mouth part gustatory sensilla, receives direct input from distant segments. Consequently, it is reasonable to think of gustatory processing as one of the major roles of the SOG. In this review, we have concentrated on the SOG in an attempt to bring together a small but widely distributed body of neuroanatomical information on gustatory processing in insects. As with our discussion of external sensilla, we concentrate on the data available from the caterpillar of *M. sexta*, the fruit fly, *D. melanogaster*, the honeybee *A. mellifera,* and large blowflies/flesh flies such as *P. regina* and *N. bullata*. The neuroanatomical information on the central processing of taste is very limited compared with that available for olfaction, yet, some patterns are beginning to emerge. Clearly, there

are numerous interesting avenues to be explored in future research.

GENERAL ANATOMY (BRAIN AND SOG)

The anatomical relationship between the brain and the SOG, particularly the tritocerebrum and the SOG, varies greatly in insects. In the four preparations considered here, the caterpillar has its SOG completely separated from the brain and connected only by circumoesophageal connectives (Fig. 2a) while the two flies and the bee show extensive fusion of these cerebral ganglia (Fig. 2b–d). Direct projections to the SOG from gustatory-mechano sensilla on various parts of the external surface and alimentary canal have been demonstrated. In the larger flies, the majority of inputs come from the cells in the labellar sensilla (Fig. 3) (Edgecomb and Murdock, 1992; Yetman and Pollack, 1986) though there is a small direct projection from the tarsal sensilla (Edgecomb and Murdock, 1992). In *D. melanogaster*, projections from sensilla on the labellum have also been described as well as ones from inside the anterior mouth parts (the labral sensory organ, the ventral cibarial sensory organ, and the dorsal cibarial sensory organ, Fig. 2c) and ones running in the maxillary nerve (Nayak and Singh, 1985; Shanbhag and Singh, 1989,1992; Singh and Nayak, 1985; Stocker and Schorderet, 1981). In the honeybee, sensory projections to the SOG have been described for the mandibular nerve (probably mechanosensory, see Fig. 5a) and the labial nerve (Rehder, 1989), as well as for some antennal sensilla (Pareto, 1972; Suzuki, 1975). In the caterpillar of *M. sexta*, sensory projections to the SOG come from the antennal, labral, mandibular, maxillary and labial nerves (Kent and Hildebrand, 1987). (Fig. 4a,b).

Separation of Mechanoreceptive and Gustatory Input

In all of the work to date, there is a less than satisfactory resolution to unambiguously separate gustatory and mechanosensory projections. However, some progress has been made on this essential first step. Feeding relies on the proper interpretation of both taste and mechanical stimuli and it is not surprising that these two modalities are well represented in the SOG, perhaps with considerable interchange that may confound anatomical separation. This is exacerbated by the fact that many insect gustatory sensilla are bimodal with a mechanoreceptive cell associated in the same sensillum with chemosensitive cells. In addition, it is theoretically possible for chemosensitive cells themselves to also convey positional information, for the contact chemoreceptive cells, by their very nature, encode mechanosensory information; in order for the cells to respond to a taste stimulus, it is necessary for the sensillum to be touching a surface, hence the activation of the taste receptor cells implies mechanosensitive inputs as well. Perhaps mechano and chemosensory cells in each sensillum send spatial information to one or more regions in the SOG. Given these realities, it is not surprising to find some additional room for interpretation of the anatomical details of taste- and mechano-innervation. Besides the bi-modal mouth part sensilla, other potential sources of mechanosensory input to the SOG include the head hair pathway (Mobbs, 1985), single modality mechanosensory sen-
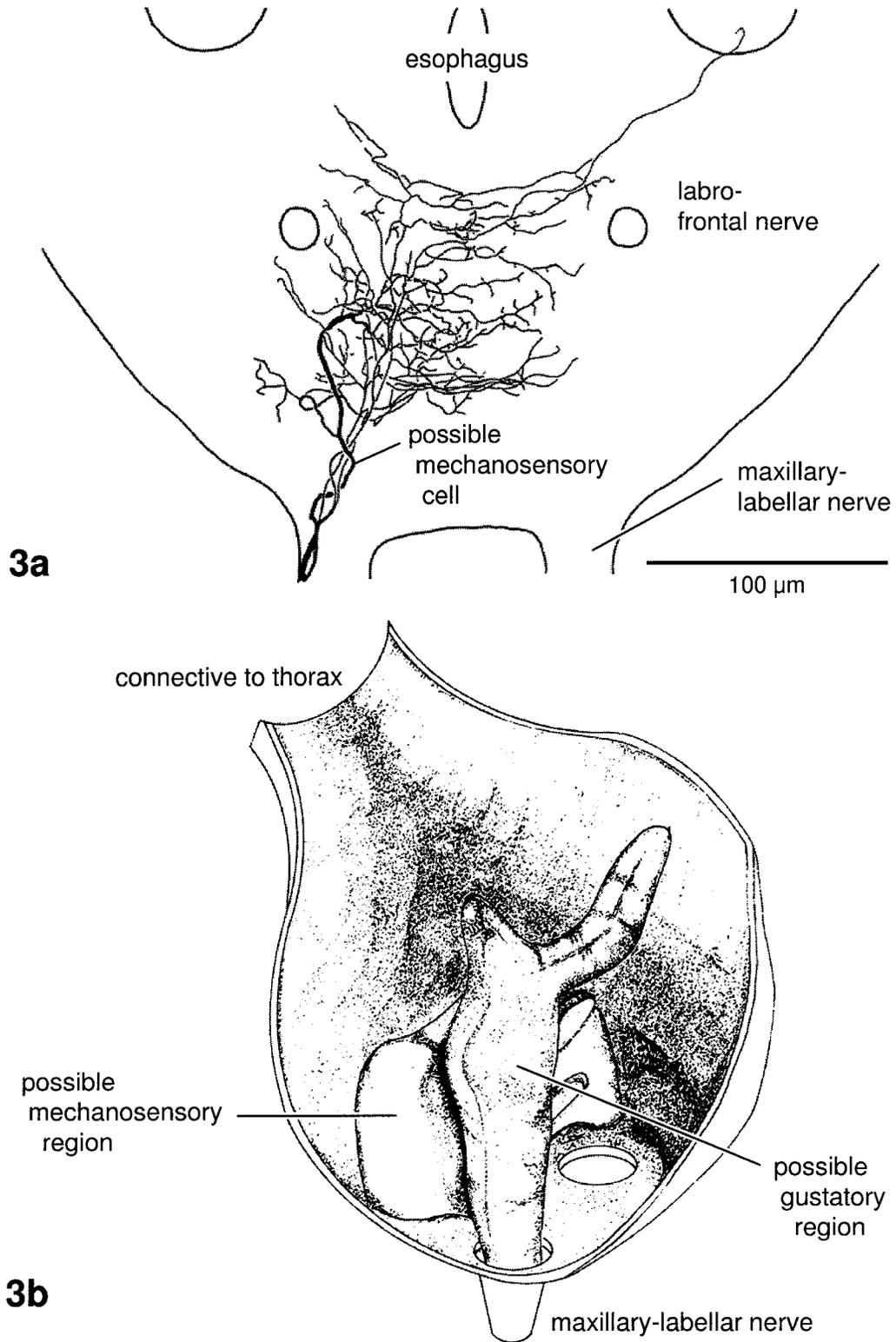
Fig. 3. Afferents from the labellar sensilla of *P. regina* project exclusively to the suboesophageal ganglion as seen in the frontal aspect reconstruction shown (**a**). Comparing fills from numerous sensilla, and interpreting the larger cells as mechanosensory suggests that the chemosensory and mechanosensory input from labellar sensilla project to distinct regions in the suboesophageal ganglion (**b**). However, three-dimensional data are not available to confirm this interpretation. Both a and b are adapted from Edgecomb and Murdock (1992).
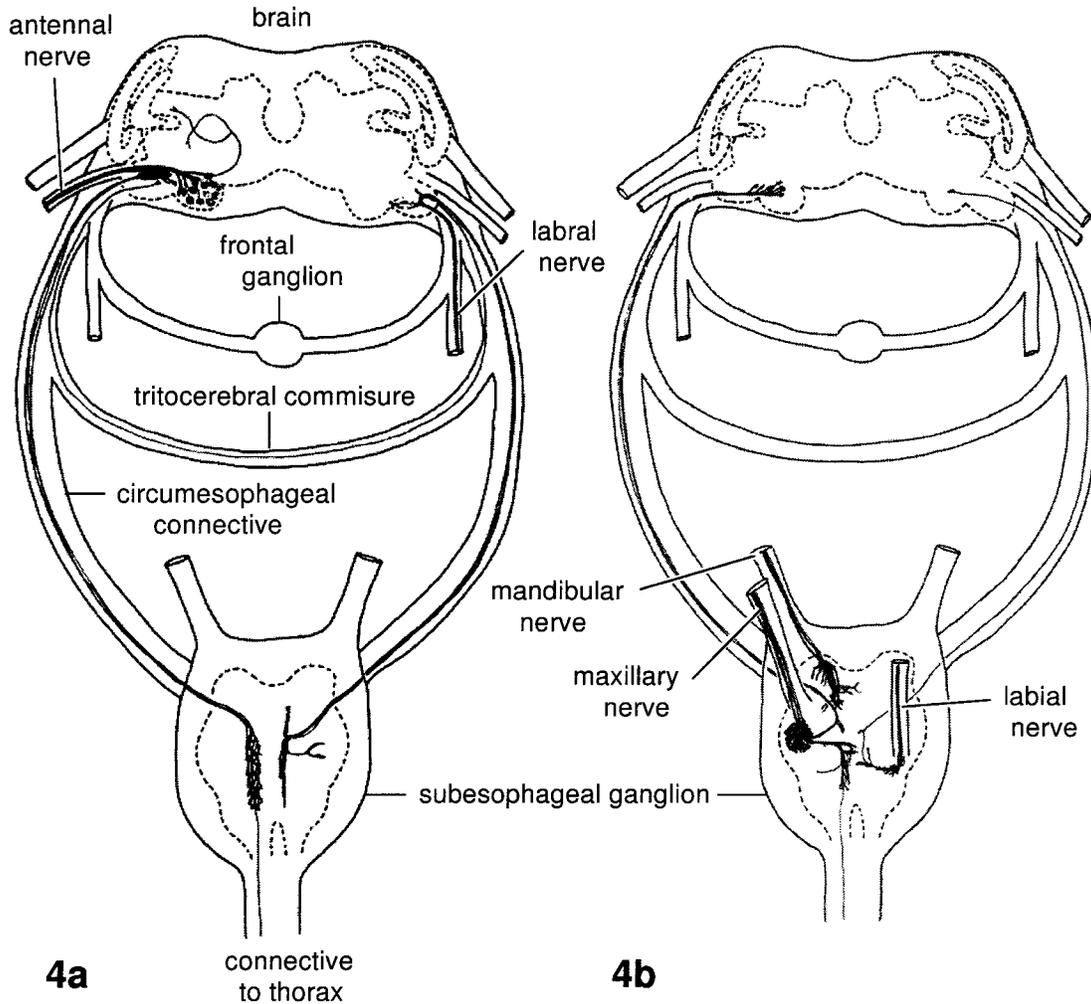
Fig. 4. Cobalt fills of all mouthpart sensilla in *M. sexta* revealed a clear partition of inputs to the suboesophageal ganglion (taste and mechanoreception), the deutocerebrum (smell), and the tritocerebrum (perhaps taste, Kent and Hildebrand, 1987; or perhaps mechanoreception, this review). **a:** Projections from fills of the antennal nerve (left side of drawing) and the labral nerve (right side of drawing). **b:** Fills from the mandibular teeth, the maxilla, and the labial plaps (mandibular, maxillary, and labial nerves). a and b adapted from Kent and Hildebrand (1987).

silla from various mouth parts, antennal mechanosensilla, and perhaps cells from thoracic segments. A clear delineation of a mechanosensory region or regions in the SOG would help greatly in separating the two modalities. In its absence, we can only summarize what is known and make suggestions for further research.

A strong case for a clear separation of mechanosensitive and gustatory input fields in the SOG is made by Edgecomb and Murdock (1992). Using cobalt fills, they were able to describe, in single labellar sensilla, a large diameter axon and one or more smaller diameter axons (Fig. 3a). They suggested that the large axon was from the single mechanosensitive cell in the treated sensillum. Summarizing results from a number of such fills, Edgecomb and Murdock suggested that there is a mechanosensory region in the SOG of *P. regina* that lies very close but just posterior to a gustatory region (Fig. 3b). Both of these regions receive massive input from the maxillary-labellar nerve and receive a large part of the gustatory and mechanosensitive input from the

labellum. Indeed, Edgecomb and Murdock's (1992) hypothesis of the separation of mechanosensory and taste projections in the SOG is supported by Murphey et al. (1989) who found that afferents of different modalities from the legs of flies project to different parts of the neuromere in the thoracic ganalion. As Edgecomb and Murdock (1992) did their work before the widespread use of confocal technology, their single-hair-fill reconstructions do not clearly separate the two modalities. Newer techniques using fluorescent dyes and computer reconstructions should allow more adequate testing of their hypothesis.

In caterpillars, represented by *M. sexta*, Kent and Hildebrand (1987) (Fig. 4a,b) clearly describe input from the mandibles that arborizes in the anterior portion of the SOG. This is almost certainly mechanosensory. There is also significant input from cells in the antennae projecting directly to the SOG and this is interpreted by Kent and Hildebrand (1987) as mechanosensory. Other inputs to the caterpillar SOG from
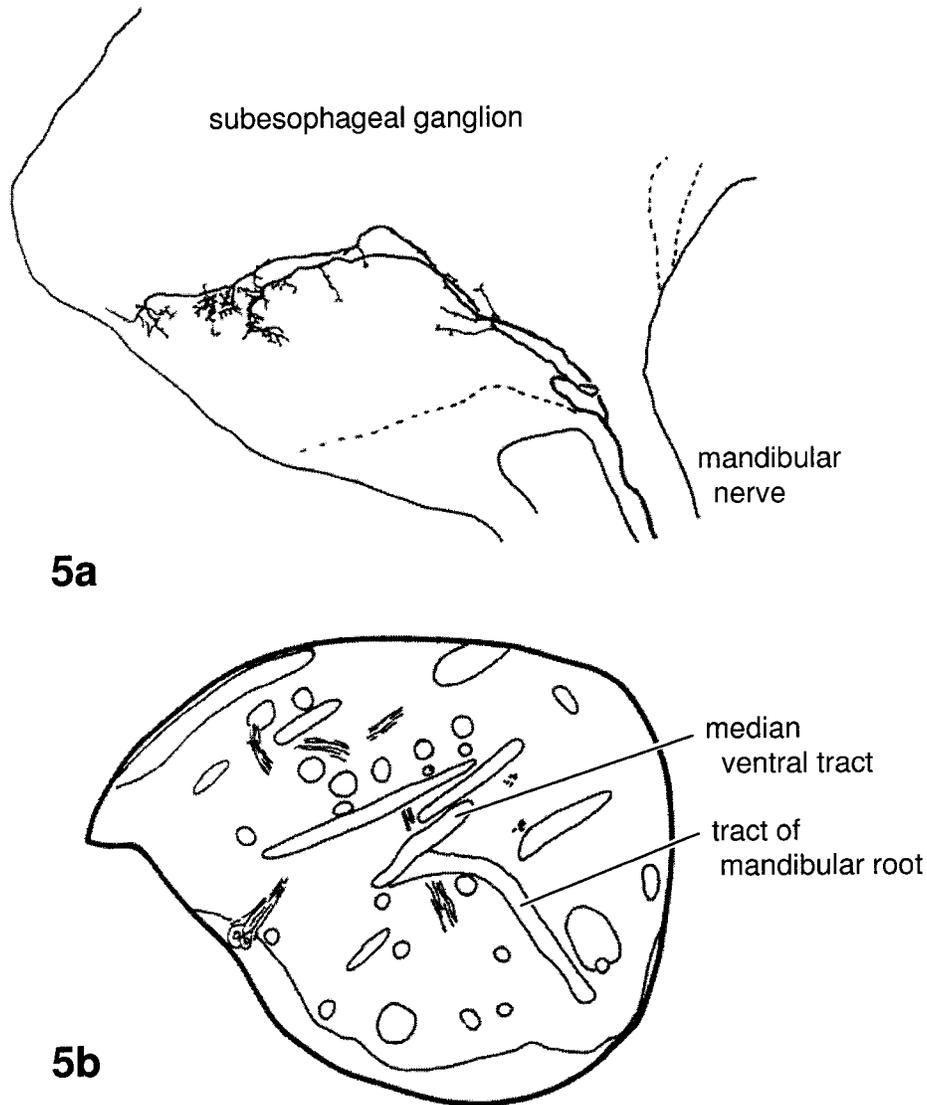
Fig. 5. Afferent projection from the mouth parts of *A. mellifera*. The honeybee sensory neuron (**a**) comes from a sensillum on the mandible base. Because a neuroanatomical map is available for the honeybee SOG (Rehder, 1988), the arborization of the sensory neuron can be accurately described using the tract reconstruction (**b**). This cell from the mandibular nerve passes through the tract of the mandibular root and into the median ventral tract before ending in the labial neuromere. a and b adapted from Rehder (1989).

labral, maxillary, and labial nerves appear to be clearly separated from each other and are probably a mixture of mechano and gustatory inputs. Uncertainty remains about small branch projections from the labral, maxillary, and labial nerves to the tritocerebrum (Fig. 4a,b). Kent and Hildebrand (1987) conclude that these are chemosensory, probably gustatory, but a case can be made for them being mechanosensory (see below). We include the tritocerebrum in our discussion of the caterpillar data because of the very close association between the SOG and tritocerebrum in the flies and honeybee.

In *D. melanogaster*, Nayak and Singh (1985) recognized seven types of sensory projections from the labellum, based on AgNO$_3$ staining. They suggest that each of the seven arborization patterns represents one of seven chemosensitive cell types with respect to sensil-

lar origin. As pointed out by Pollack and Balakrishnan (1997), there is little evidence to support the assumption that only gustatory and not mechanosensory fibres were selected by the silver stain. Like blowflies and flesh flies, labellar sensilla of *D. melanogaster* are biomodally innervated for taste and mechanoreception (Falk et al., 1976). It seems more likely that the silver-stained projections shown by Nayak and Singh (1985) represent a selection of chemo- and mechanosensitive fibres and that, as in the larger flies, more work is needed to anatomically separate mechano- and chemo-processing areas in the SOG.

The anterior alimentary canal of *D. melanogaster* has three groups of sensilla (Stocker and Schordert, 1981) the analogues of which have not yet been described in blowflies and flesh flies, though they have been sought (Dethier, 1976). These inner labral (epipharyngeal) and
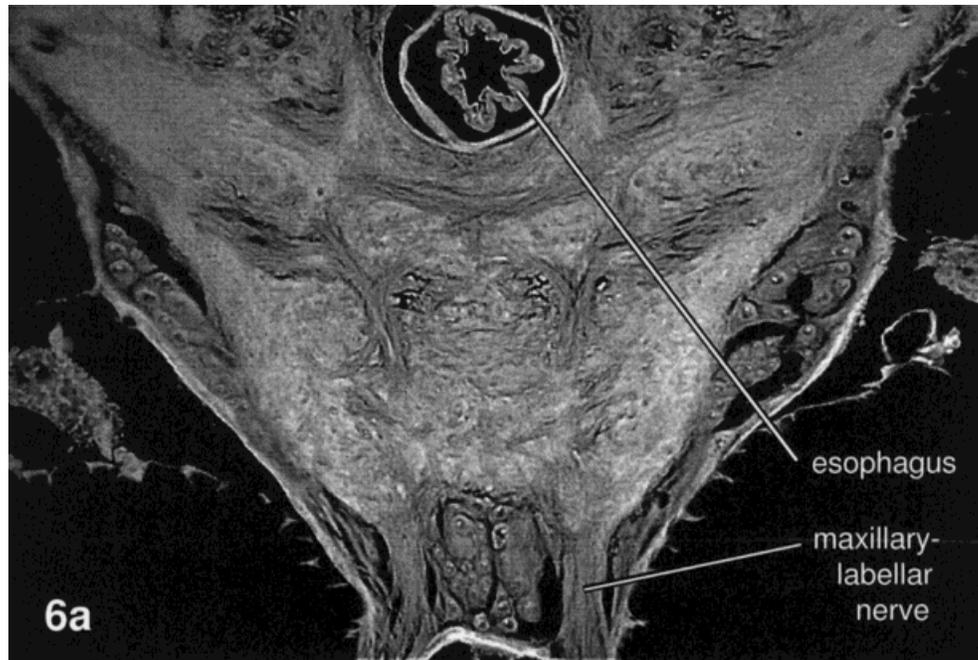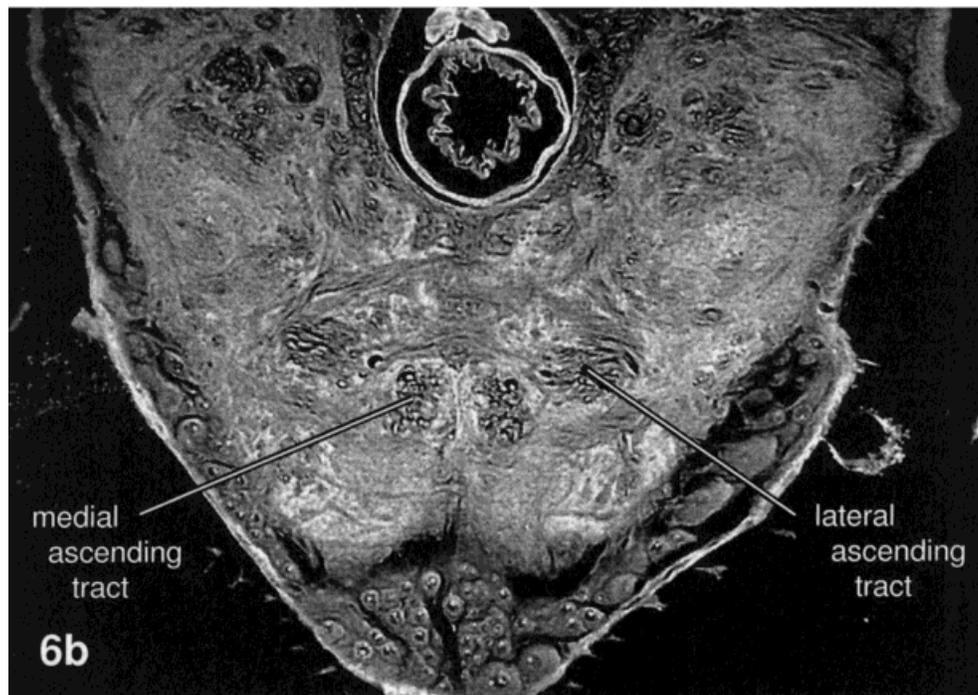
Fig. 6. Frontal sections (8 µm) of the SOG in *N. bullata*, as revealed by Crystal Violet-Pyronin Y staining and viewing under epi-fluorescence. See text for discussion. **a:** Section at level of the maxillary-labellar nerve. **b:** Posterior to a.

cibarial sensilla are primarily single celled and, as revealed by cobalt filling, they project via pharyngeal and accessory pharyngeal nerves to the tritocerebrum

(Stocker and Schorderet, 1981). Epipharyngeal sensilla in *M. sexta* larvae project, via the labral nerve, to both the SOG and the tritocerebrum (Kent and Hildebrand,

7
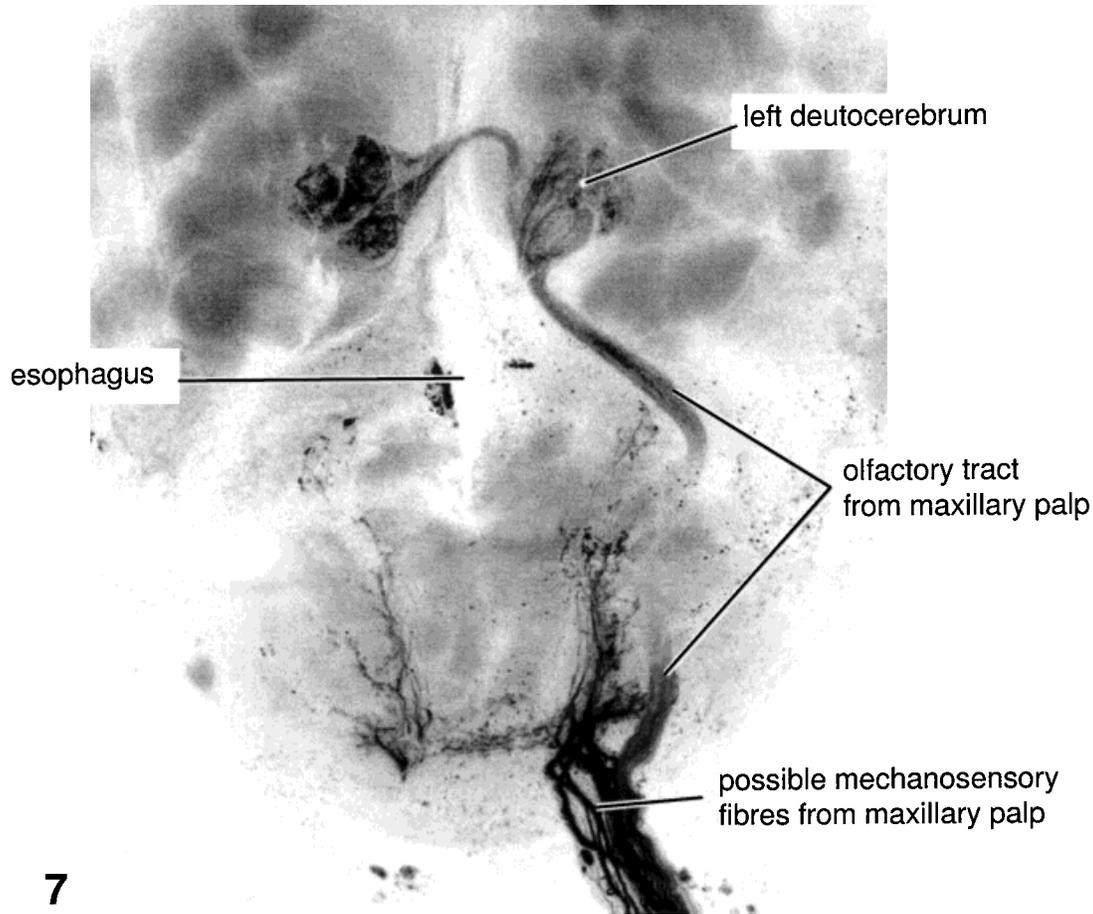
Fig. 7.   Dextran-rhodamine fill of maxillary-labellar nerve showing olfactory tract from the maxillary palp that carries olfactory input to the duetocerebrum. Because of the nature of the preparation, we are uncertain if the projections to the SOG shown are from gustatory or mechanosensory cells. This is a composite of two confocal reconstructions made of overlapping scans of a whole mount preparation.

1987). While there may indeed be a gustatory association region in the tritocerebrum serving the labral appendages, this region appears to be very close to the antennal mechanosensory region described in various insects. For example, mechanosensory projections to this region are know from head hairs of locusts and interommatidial sensilla of the honeybee (Mobbs, 1985), and from antennal mechanosensilla in moths (Homberg et al., 1989). The region is often referred to as the antennal mechanosensory centre (Strausfeld, 1976). More detailed studies are needed before we can be sure that there is a gustatory processing centre in the tritocerebrum.

Anatomy of the Sub-Oesophageal Neuropil

The complexity of the SOG, due to the various degrees of fusion of the three mouth part neuromeres, has hindered our understanding of this important processing centre. The most comprehensive summary of the anatomy of this ganglion is that of Altman and Kien (1987), where the emphasis is on motor control in the locust. However, our understanding of the taste inputs to the locust SOG is very poor and the relative projection patterns of the hundreds of gustatory and mechanosensitive cells on locust mouth parts is practically unknown. As the large body of locust motor control work has shown, a full understanding of the general anatomy of relevant neuropils is a pre-requisite to a complete interpretation of single-cell anatomical and physiological results.

The work on gustatory and mechanosensory projections to the SOG of flies and caterpillars described in previous sections suffers from inadequate descriptions of the complex neuropil. None of the detailed patterns described is placed in the context of various neuropile landmarks such as tracts and commissures. In contrast to flies and caterpillars, general SOG neuropil anatomy of the honeybee has been well described (Rehder, 1988). Rehder (1989) also described some of the sensory projections to the SOG from mandibular and labial nerves. While the bee literature lacks detailed information on individual gustatory cell projections, Rehder's illustration, reproduced as Figure 5a and b, shows the level of general anatomical information that is needed to fully use the single-cell fills and reconstructions that are possible (Mitchell and Itagaki, 1992).

Recognizing this need, we have made some progress toward describing the SOG neuropil in the flesh fly

8a

8b sucrose-sensitive local interneuron

8c same cell as above showing bi-layered arborization
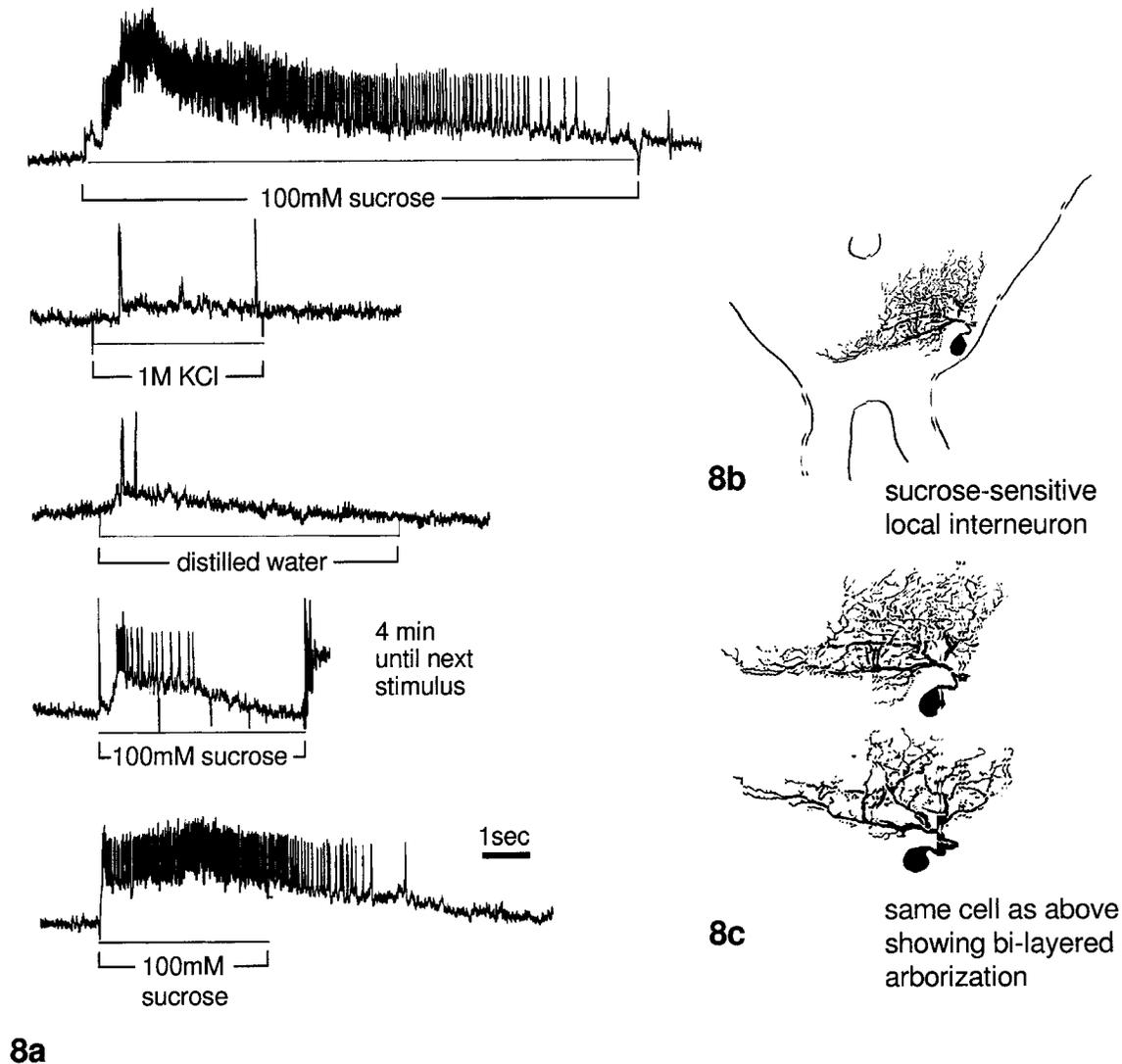
Fig. 8. Representative sucrose-sensitive interneuron in the SOG of *N. bullata*. **a:** Several labellar sensilla were simultaneously stimulated with one of 100 mM sucrose, 1M KCl, and distilled water and responses of an interneuron are shown. The response to sucrose adapted (trace 4) but recovered after 4 min (trace 5). **b:** The relative position of the recorded interneuron in the SOG is shown in the top reconstruction. **c:** The bi-layered form of this cell is depicted in the bottom two drawings. These two layers may represent arborizations in the maxillary and labial neuromeres, respectively, but more anatomical information is needed to confirm this. Adapted from Mitchell and Itagaki (1992).

N. bullata. Here, as in *D. melanogaster*, there is no mandibular neuropil, and the maxillary and labial neuropils are highly integrated (also see Strausfeld, 1976, for *M. domestica*). Figure 6a,b shows the *N. bullata* SOG in the region where the maxillary-labellar nerve emerges. This nerve carries olfactory information from the maxillary palpi in a lateral bundle and gustatory and mechanosensory fibres from the labellar lobes in a medial bundle (Fig. 7). This olfactory tract passes directly through the SOG to arborize in some of the olfactory glomeruli in the deutocerebrum. This major tract has also been described in *D. melanogaster* (Singh and Nayak, 1985). Mechanosensory fibres from the maxillary palpi are also carried in this nerve and they arborize in the SOG (Singh and Nayak, 1985). Whether this maxillary mechanosensory input overlaps with labellar mechanosensory input is yet to be determined.

The large median and lateral ascending tracts of the SOG (Fig. 6a,b) are likely homologous with the median ventral tracts (MVT) described in the honeybee by Rehder (1988). Our designation of these tracts as ascending follows Strausfled's 1976 description of similar tracts in *M. domestica*. Rehder suggests some correspondence between the MVT in the honeybee and comparable tracts in *M. sexta* larvae described by Kent and Hildebrand (1987). Based on locust data (Tyrer and Gregory, 1982) and on his honeybee data, Rehder (1988) also points out that fibres from mouth part nerves and higher brain centres converge in the region of the MVT, making this region a likely place for major sensory

integration. This area deserves special attention in future attempts to describe the neuroanatomy of gustatory processing. If it is primarily an ascending tract as Strausfeld (1976) suggests, then it may be a major centre for sending partly processed gustatory information forward to the brain.

Interneurons of the SOG Gustatory System

Despite the probable need to send gustatory information to higher centres, it must be remembered that the proboscis extension reflex (PER) seen in flies and bees implies local neural circuitry that can lead from taste input to behaviour with fairly limited processing. That is, there are probably interneurons that directly link taste input and motor output. Mitchell and Itagaki (1992) described several local SOG interneurons that responded when the labellum of *N. bullata* was stimulated with sucrose, water, or salt. It was possible to find cells specific to these modalities, but the limited number of LY fills did not allow a morphological classification of these cells. An example of one of these interneurons sensitive to sucrose but not to salt is shown in Figure 8. Thus, for insect gustation, we still have no morphologically identified cell or cells that can be seen as part of the taste processing system. With more intracellular work we hope morphologically identified cell types will emerge, which, like the primary afferents, should be placed in the general context of the SOG anatomy. By contrast, physiological response characteristics of interneurons are recognizable using intracellular approaches and simple stimuli such as sucrose, water, salt, and touch (Mitchell and Itagaki, 1992; Rivet, unpublished data).

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