

An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths

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Summary. In the hawkmoth, *Manduca sexta*, the third segment of each labial palp contains a pit, which houses a densely packed array of sensilla. We have named this structure the labial pit organ (LPO). The sensilla within the pit are typical of olfactory receptors, characterized by a grooved surface, wall pores, and pore tubules. Axons arising from receptor cells that innervate these sensilla project bilaterally to a single glomerulus in each antennal lobe. We have compared this central projection with that in three other species of *Manduca* (*M. quinquemaculata*, *M. dilucida*, and *M. lanuginosa*) and in the silkmoths *Antheraea polyphemus* and *Bombyx mori*. A bilateral projection to a single glomerulus in each antennal lobe is present in all cases. We suggest that the LPO serves as an accessory olfactory organ in adult Lepidoptera.

Key words: Antennal lobes – Labial palps – Labial pit organ – Olfactory system – *Manduca sexta* (Insecta)

Olfaction in insects is associated primarily with the antennae. In many insects, however, mouthpart appendages, such as the labial and maxillary palps, also may bear olfactory organs (Wigglesworth 1972). In adult Lepidoptera, the terminal segment of each labial palp contains numerous sensilla that lie within a depression, groove, or pit (Hicks 1860; vom Rath 1888). Because a tactile or contact-chemosensory function seemed unlikely for sensilla recessed within a pit, vom Rath deduced that they were olfactory sensilla.

Snodgrass (1935) referred to the sensilla within the pit in each labial palp of *Pieris rapae* as coeloconic sensilla, peg organs sunken into depressions of the body wall. Coeloconic sensilla have been found to house chemoreceptors or chemoreceptors combined with a thermoreceptor or hygroreceptors combined with a thermoreceptor (Altner et al. 1983 a).

A hygroreceptive function was suggested for the sensilla on the labial palps of keratophagous Lepidoptera based upon a correlation between the number of sensilla and the humidity of the oviposition site chosen by the females (Faucheux and Chauvin 1980).

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Recently, the sensilla in the “labial pit organs” (LPOs) of *Pieris rapae* and *Pieris brassicae* were examined electrophysiologically and ultrastructurally. The structure of the sensilla was found to be typical of olfactory sensilla, characterized by wall pores and pore tubules. Sensory cells associated with the sensilla responded to natural complex odors, including odors of host plants and conspecific animals. No evidence was obtained for thermoreceptive or hygroreceptive functions (Lee et al. 1985).

While central projections of antennal axons have been traced in several species of insects (bees: Pareto 1972; Suzuki 1975; cockroaches: Boeckh et al. 1970; flies: Stocker et al. 1983; moths: Camazine and Hildebrand 1979; Camazine et al., in preparation), input to the brain and subesophageal ganglion (SEG) from other gustatory and olfactory organs has been reported only in *Drosophila* (Stocker and Shorderet 1981; Nayak and Singh 1985) and larval *Manduca* (Kent and Hildebrand 1986).

We have examined the LPO in *Manduca sexta* and have traced the central projections of axons arising from it. In addition, we have compared the projections of these axons in three other species of *Manduca* and in the silkmoths *Antheraea polyphemus* and *Bombyx mori*. In all cases, axons from the labial palp project to a single glomerulus within each antennal lobe, a region of the deutocerebrum known to be the primary integration center for olfactory information from the antenna. Such a projection supports the idea that sensilla within the LPO are olfactory receptors. A preliminary account of some of our findings has appeared elsewhere (Harrow et al. 1983).

Materials and methods

Manduca sexta and *M. quinquemaculata* (Lepidoptera: Sphingidae) were reared on artificial diet (Bell and Joachim 1976) on a long-day photoperiod regimen (17 h light, 7 h dark) at 26° C and approximately 60% relative humidity as described previously (Sanes and Hildebrand 1976). Pupae of *M. dilucida* and *M. lanuginosa* from Santa Rosa National Park, Costa Rica, were obtained from Dr. D. Janzen, and pupae of the laboratory-reared saturniids *Antheraea polyphemus* and *Bombyx mori* were provided by Prof. K.-E. Kaissling and Dr. R. O’Connell, respectively.

Labial palps were fixed in alcoholic Bouin’s solution (Humason 1972) or buffered 4% paraformaldehyde, dehy-

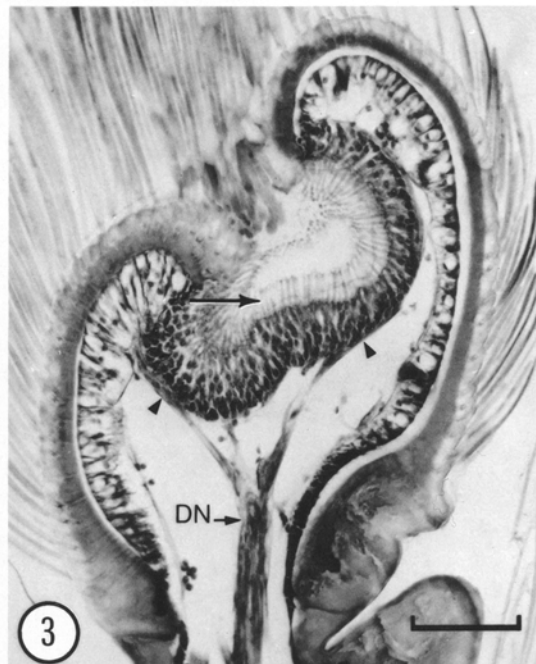


Fig. 1. Anterior view of the head of *M. sexta*. The scales from one labial palp have been removed to reveal the third (terminal) segment (arrow), which exhibits a depression and pore leading to the labial pit organ (LPO). Scale bar = 1000 μm

Fig. 2. Scanning electron micrograph of the terminal segment of a labial palp of *M. sexta*. The segment was bisected to reveal the flask-shaped LPO containing numerous sensilla (arrow). Courtesy of Drs. A. Grant and M.S. Mayer. Scale bar = 100 μm

Fig. 3. 8- μm thick paraffin section through the LPO of *M. sexta* showing the sensory neurons and supporting cells (arrowheads) beneath the sensilla (arrow). Axons from the sensory neurons collect into a dorsal nerve bundle (DN). Scale bar = 100 μm

drated through graded alcohol solutions, embedded in Paraplast Plus (Sherwood Medical Industries, St. Louis, MO), and sectioned at 8 μm . Sections were stained with Mallory-Heidenhain stain (Humason 1972) and mounted under coverslips in Permount or Canada Balsam (Fisher Scientific Co., Fair Lawn, NJ).

Axons from sensilla within the LPO were stained by affixing the moth to a wax pedestal and inserting a micropipette filled with a dilute solution of cobaltous-lysine complex (Lazar 1978) into the cut end of the terminal segment. The micropipette was left in place for three days at 4° C. Subsequently, brains were excised, the cobalt was precipitated with aqueous hydrogen sulfide, and the brains were fixed in Carnoy's or Bouin's solution (Humason 1972). Whole mounts or 8- μm thick sections were processed according to modifications of Timm's silver intensification method (Bacon and Altman 1977; Tyrer and Bell 1974; Matsumoto and Hildebrand 1981; Kent and Hildebrand 1986).

In other preparations, axons from the LPO sensilla were stained along with other labial nerve fibers. The brain was excised with the labial nerves attached and placed in a Petri dish coated with Sylgard (Dow-Corning). The first labial nerve (Eaton 1974) was draped over a petroleum-jelly bridge into a solution of cobaltous-lysine complex or Lucifer Yellow CH (3% aqueous) (Stewart 1978). The brain was immersed in a drop of saline (Cherbas 1973) and incubated at 4° C for 5–8 h. When a cobalt solution was used, the tissue was then processed as described above. When Lucifer Yellow was used, the tissue was fixed in buffered 4% paraformaldehyde, dehydrated, cleared, and examined with a compound microscope equipped for epifluorescence.

Results

The labial palps of *M. sexta* are three-segmented appendages that are covered with scales and project upward at the sides of the proboscis. The terminal segment of each palp is small in comparison to the other two segments, about 400 μm in length and diameter, and can be seen only if the surrounding scales are removed (arrow in Fig. 1).

Scanning electron micrographs and histological sections through the terminal segment of the labial palp of *M. sexta* revealed a pit, the labial pit organ (LPO), that houses a densely packed array of sensilla (arrow in Figs. 2, 3). Beneath the sensilla is an epidermis containing sensory neurons and supporting cells (arrowheads in Fig. 3). Axons arising from the sensory neurons collect into a dorsal nerve bundle (DN in Fig. 3) that joins a ventral nerve bundle to form the first labial nerve (ON3, nomenclature after Nüesch 1957; Eaton 1974).

The opening of the pit is on the forward-facing surface of the terminal segment and is about 30 μm in diameter. The pit is flask shaped, with a narrow neck and wider inner cavity that reaches 200 μm in diameter in one direction and 300 μm in depth. Based on counts of sensilla in 8- μm thick sections through the LPO, we estimate that there may be as many as 1750 sensilla in the LPO of *M. sexta*. No sexual dimorphism is evident.

In high-magnification scanning electron micrographs, the sensilla appear to have an irregularly grooved surface, as if formed by numerous fingers or flaps (Fig. 4). The sensilla are about 30 μm in length and 7 μm in diameter. Transmission electron microscopy revealed that the wall

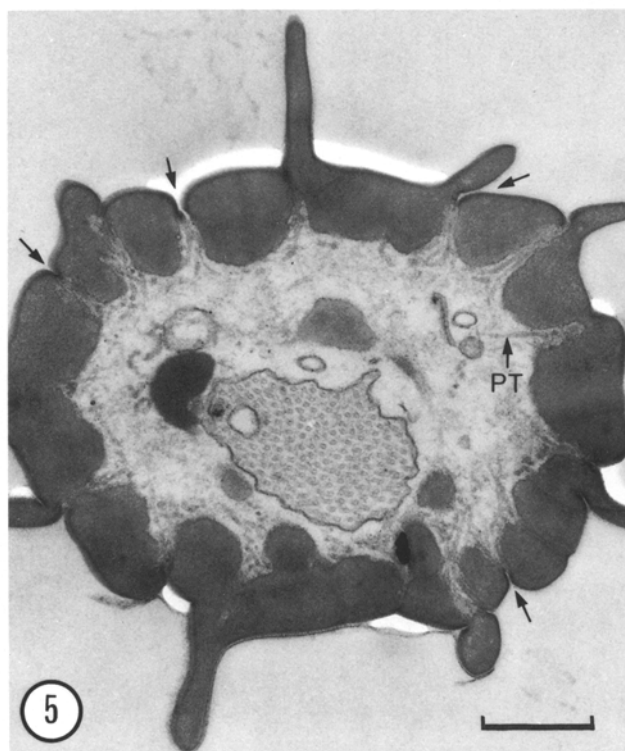
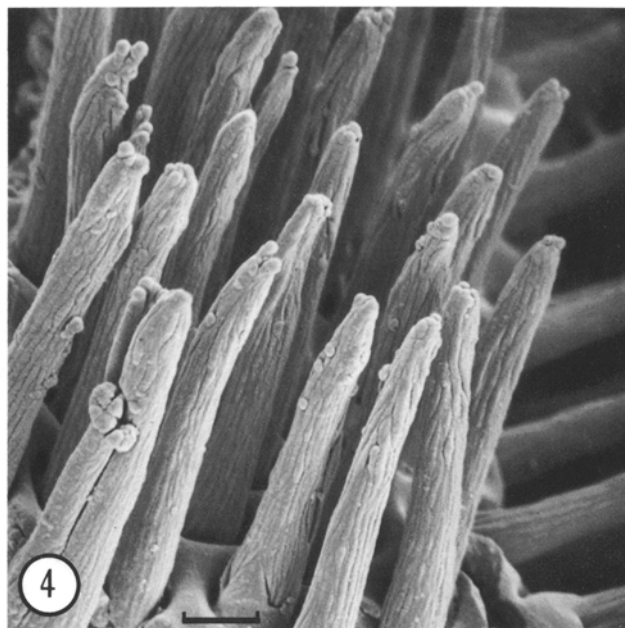


Fig. 4. Scanning electron micrograph showing the irregularly grooved surface of the sensilla within the LPO of *M. sexta*. Courtesy of Drs. J.-K. Lee and H. Altner. $\times 2000$. Scale bar = 5 μm

Fig. 5. Transmission electron micrograph through the proximal portion of a sensillum from the LPO of *M. sexta*. Numerous wall pores (arrows) pierce the irregularly grooved surface of the sensillum. Pore tubules (PT) extend into the outer lymph cavity that surrounds the dendrite, which is cylindrical in this proximal section of the sensillum. Courtesy of Drs. J.-K. Lee and H. Altner. $\times 30000$. Scale bar = 0.5 μm

of the sensillum is pierced by numerous pores (arrows in Fig. 5) as in *Pieris* (Lee et al. 1985). Pore tubules (PT in Fig. 5) extend into the outer lymph cavity surrounding the dendrite, which is cylindrical in its proximal portion (Fig. 5)

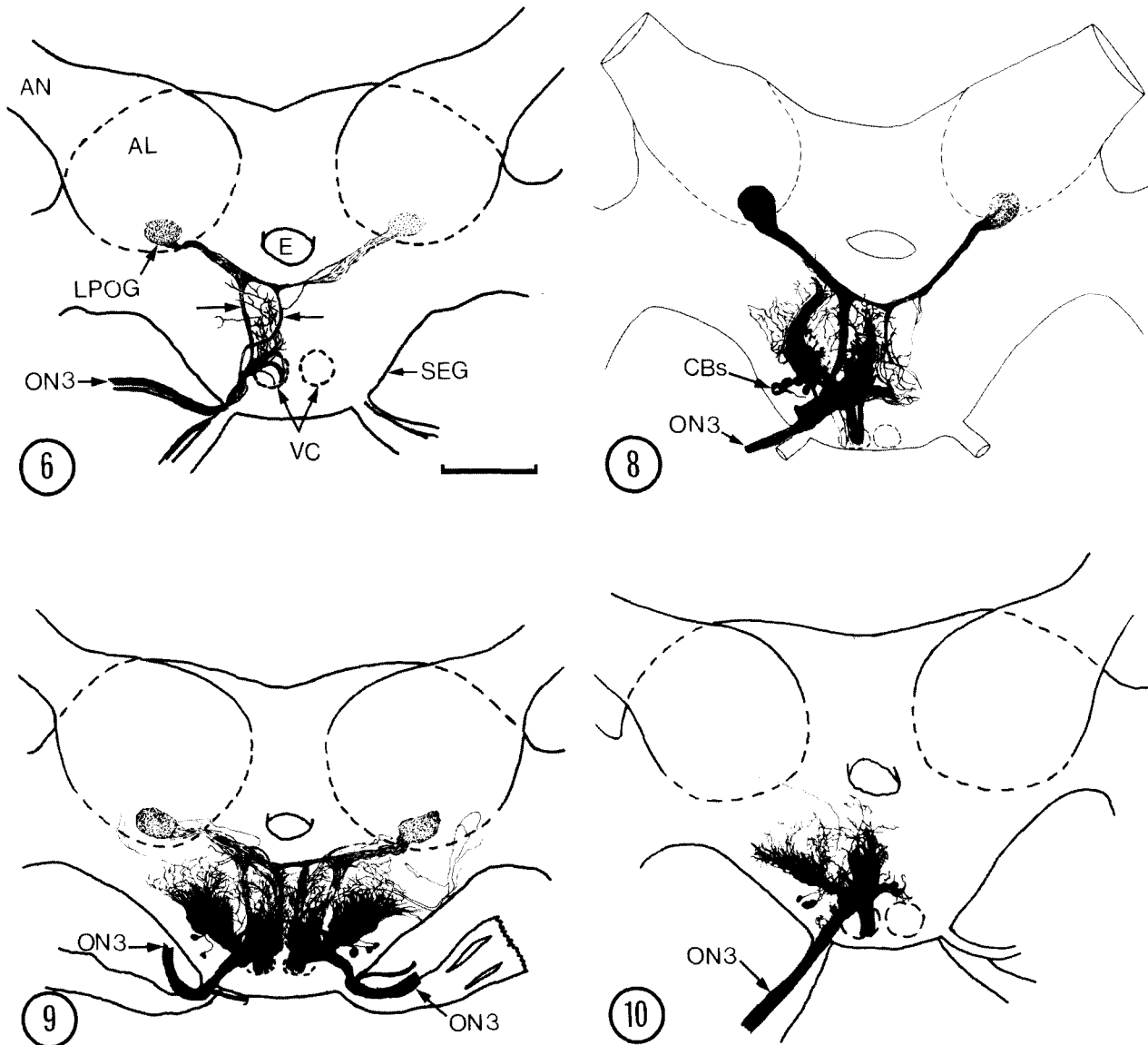


Fig. 6. Camera lucida drawing of an anterior view of the brain of *M. sexta* showing fibers that stained after cobalt solution was applied to the cut end of the terminal segment of a labial palp. Fibers enter the SEG via the first labial nerve (*ON3*) and extend dorsally in two tracts (arrows) that bifurcate ventral to the esophageal foramen (*E*). Many axons project ipsilaterally and somewhat fewer project contralaterally to a single glomerulus (*LPOG*) in the posteroventral region of each antennal lobe (*AL*). Some sparse arborizations appear in the SEG, and some axons leave the SEG through the ventral connective (*VC*). *AN*, antennal nerve. Scale bar = 100 μ m

Fig. 8. Camera lucida drawing of an anterior view of the brain of *M. sexta* showing the staining pattern achieved by staining all axons in the first labial nerve (*ON3*). Cell bodies (*CBs*) are probably motor neurons that innervate musculature of the labial palp. Scale same as in Fig. 6

Fig. 9. Camera lucida drawing of a frontal view of the brain of *M. sexta* showing the overlap in the *LPOG* of projections from axons of the right and left labial nerves (*ON3*). Scale same as in Fig. 6

Fig. 10. Camera lucida drawing of a frontal view of the brain showing the staining pattern obtained by staining axons in the first labial nerve (*ON3*) one week after the terminal segment of the labial palp had been ablated. Neither the projections to the *LPOG* nor the characteristic tracts in the SEG were stained. Scale same as in Fig. 6

but lamellated and folded in its distal portion as shown in *Pieris* (Lee et al. 1985). Approximately 5–10% of the sensilla house two sensory cells and the rest house only one (Altner, personal communication).

Axons, stained from the cut end of the third segment with a solution of cobaltous-lysine complex, enter the SEG via the first labial nerve (*ON3* in Fig. 6). Some fibers have

arborizations on the ipsilateral side of the SEG. Other fibers form two tracts, on the ipsilateral side of the SEG, which travel in a dorsal direction (arrows in Fig. 6). As the fibers approach the esophageal foramen, the tracts bifurcate and extend fibers to both ipsilateral and contralateral antennal lobes. Arborizations of these fibers are confined to a single glomerulus (*LPO-glomerulus*, *LPOG* in Fig. 6) near the lat-

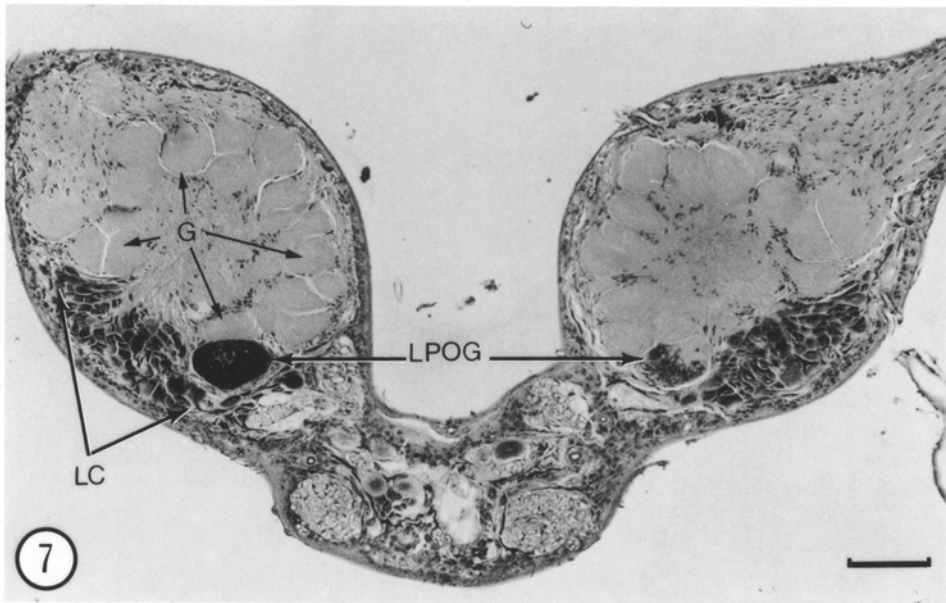


Fig. 7. 8- μ m thick frontal paraffin section through the brain of *M. sexta* showing the cobalt-stained LPOG in its characteristic position in the posteroventral region of the AL. The LPOG ipsilateral to the stained nerve (left) lies between the lateral cluster of neuronal somata (LC) and the array of "ordinary" glomeruli (G). The contralateral LPOG (right) occupies a similar position but is less densely stained. Scale bar = 100 μ m

eral edge of the posteroventral region of each antennal lobe. Histological sections through the region of the antennal lobe containing the stained LPO-glomerulus demonstrate the characteristic position of the LPO-glomerulus between the lateral cluster of antennal-lobe somata (LC in Fig. 7) and the array of "ordinary" glomeruli (G in Fig. 7).

Staining all axons in the first labial nerve revealed a similar projection pattern and, in addition, included other branching patterns within the SEG as well (Fig. 8). Stained cell bodies (CBs in Fig. 8) probably belong to motoneurons that innervate the musculature of the labial palps.

A single glomerulus in each antennal lobe stained after both right and left first labial nerves had been filled with cobalt, establishing that each LPO contributes sensory input to one and the same glomerulus (the LPO-glomerulus) in each antennal lobe (Fig. 9). The staining pattern appears to be identical in males and females.

To confirm that the axons projecting to each antennal lobe arise from sensilla within the terminal segment, we ablated the terminal segment one week prior to staining axons in the first labial nerve. In these preparations, neither the two characteristic tracts in the SEG nor the projections to the antennal lobes were observed (Fig. 10). By contrast, the pattern of stained neurites in the SEG is relatively normal. These stained processes probably arise from other sensory receptors in the labial palp and from motoneurons innervating musculature of the labial palps. Although sparse arborizations were observed in the SEG after axons were stained from the cut end of the terminal segment (Fig. 6), we could not determine whether these sparse arborizations were present in the SEG after the terminal segment had been ablated. Thus, we are not certain that sensory axons from the LPO have arborizations in the SEG as well as in the antennal lobes.

Staining the first labial nerves in *M. quinquemaculata*, *M. dilucida*, and *M. lanuginosa* also demonstrated bilateral projections to each antennal lobe (Figs. 11–13). In *Antheraea* and *Bombyx*, bilateral projections to each antennal lobe were observed, but the "glomerular" arborizations (arrows in Figs. 14, 15) were small in comparison to those in *Man-*

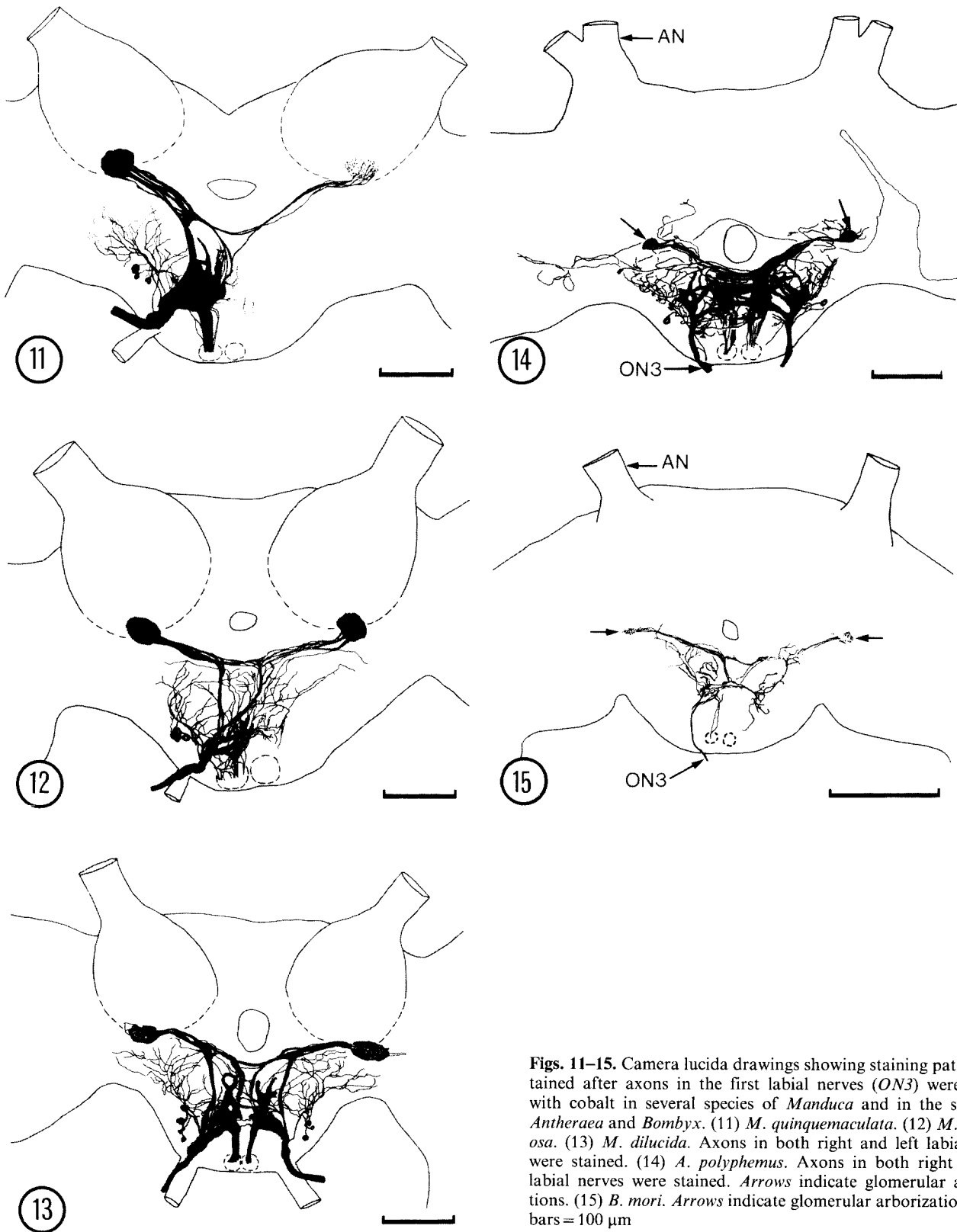
duca. Histological sections through the labial palps of *Antheraea* and *Bombyx* revealed a shallow depression in place of a pit at the tip of each palp. In *Antheraea*, each LPO contains approximately 40 sensilla (Fig. 16).

Discussion

Pit organs or depressions of the cuticle containing a number of sensilla have been reported in the labial palps and antennae of several species of insects in addition to Lepidoptera. Certain flies have pit organs in their antennae (Röhler 1906; Liebermann 1926; Kaib 1974), and in the blowfly these sensilla have been shown to be olfactory (Kaib 1974). Similarly, the antenna of the locust contains small pit organs (*sensilla coeloconica*), which house single sensilla that also have been shown to have an olfactory function (Boeckh 1967). The terminal segments of the labial palps in representatives of certain families of Neuroptera possess pit organs densely packed with sensilla (Eisner 1953).

The labial pit organs in *M. sexta* were previously described by Vande Berg (1968). They resemble those described in certain other Lepidoptera, with a narrow pore guarded by scales and a larger inner cavity filled with sensilla (Hicks 1860; vom Rath 1888; Hsü 1938; Lee et al. 1985). In other species of Lepidoptera, the pit may be reduced to a mere depression, or there may be no pit at all (Hicks 1860). In some species of keratophagous moths, the sensilla are not collected in one large pit but are isolated in their own small pits (Faucheux and Chauvin 1980). Individuals of one species have just a single sensillum on the labial palp (Faucheux and Chauvin 1980).

The ultrastructural characteristics of the sensilla within the LPO of *Manduca* are nearly identical to those described in *Pieris* (Lee et al. 1985). In both *Manduca* and *Pieris*, the sensilla are characterized by grooved surfaces, wall pores, pore tubules, and lamellated dendritic outer segments. The presence of wall pores and pore tubules is typical of olfactory receptors (Altner 1977a, 1977b; Altner and Prillinger 1980). Lamellated dendritic outer segments have been described in the antennal sensilla of cave beetles (Cor-



Figs. 11–15. Camera lucida drawings showing staining patterns obtained after axons in the first labial nerves (*ON3*) were stained with cobalt in several species of *Manduca* and in the silkmoths *Antheraea* and *Bombyx*. (11) *M. quinquemaculata*. (12) *M. lanuginosa*. (13) *M. dilucida*. Axons in both right and left labial nerves were stained. (14) *A. polyphemus*. Axons in both right and left labial nerves were stained. *Arrows* indicate glomerular arborizations. (15) *B. mori*. *Arrows* indicate glomerular arborizations. Scale bars = 100 μ m

bière-Tichané 1971; Corbière-Tichané and Bermond 1972). The sensilla of the cave beetle are poreless, however, and physiological evidence suggests that the sensory cells with lamellated outer segments are thermoreceptive (Altner et al. 1978, 1981, 1983b; Loftus and Corbière-Tichané 1981). Lamellated dendritic outer segments have also been reported

in sensilla containing wall pores. These occur on the antennae and palps of certain Diptera (Lewis 1971; McIver 1972; Hechler and Rühm 1983) and are assumed to belong to chemoreceptors (Lee et al. 1985). As in *Pieris*, therefore, the sensilla within the LPO of *Manduca* probably respond to olfactory cues. Preliminary electrophysiological results

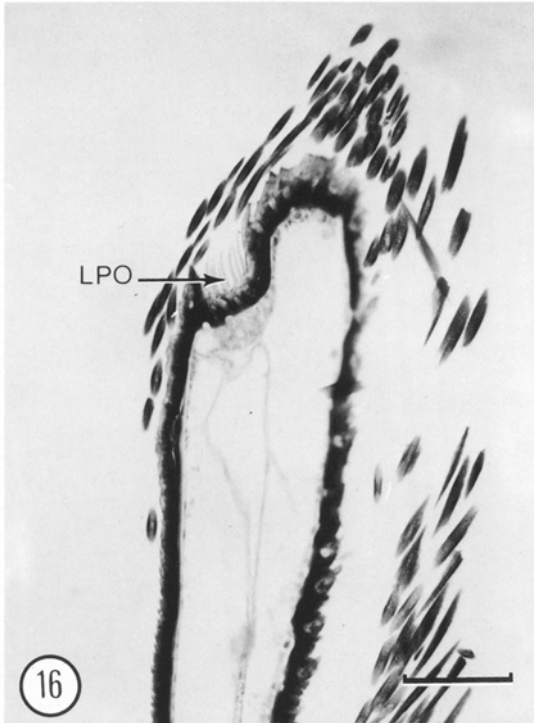


Fig. 16. 8- μ m thick paraffin section through the LPO of *A. polyphemus*. The LPO is small in comparison to the LPO of *Manduca* and contains fewer sensilla. Scale bar = 100 μ m

from our laboratory support an olfactory function (Harrow et al. 1983). LPO receptors responded to volatile constituents of plant extracts – perhaps organic substances, CO₂, or water vapor.

The LPO of *M. sexta* contains many more sensilla (up to 1750) than the LPOs that have been described in other species. The LPO of *Pieris* contains about 80 sensilla (Lee et al. 1985). Different species of keratophagous moths possess between one and 70 sensilla (Faucheux and Chauvin 1980). The large size of *Manduca* alone cannot account for the difference in number of sensilla because *Antheraea*, a comparably large moth, possesses only about 40 sensilla in its LPO.

The total number of chemosensilla and chemosensitive neurons in the mouthparts of certain Diptera has been correlated with the variety of the insects' sources of food. For example, the blowfly *Phormia*, which uses a wide range of food sources, has much larger absolute numbers of chemosensilla on its mouthparts than does the tsetse fly, which feeds only on blood (Rice et al. 1973). The black fly, which is intermediate in number of sensilla on its mouthparts, is also intermediate in number of food sources (Sutcliffe and McIver 1982).

While *Manduca* and *Antheraea* are both oligophagous as larvae, *Manduca* continues to feed as an adult, but *Antheraea* does not. Perhaps input from a large number of sensilla in the LPO is important for both feeding and non-feeding behavior (such as selecting a site for oviposition or mating) in *Manduca*, while a smaller number is sufficient for non-feeding behavior alone in *Antheraea*. The smaller glomerular projection in *Antheraea* may reflect the smaller number of sensilla.

A difference in the number of sensilla in the labial palps of several species of keratophagous moths has been corre-

lated with differing degrees of humidity of the oviposition site. These moths do not feed as adults but select oviposition sites such as wool or skin. Moths choosing humid sites have relatively many more sensilla (70) than those choosing dry sites or showing no preference (often only 1 sensillum) (Faucheux and Chauvin 1980). Nevertheless, ablation experiments demonstrated that the antennae were more important than the labial palps in the selection of a humid oviposition site (Faucheux 1982). Faucheux suggested that the function of the LPOs in these species may be synergistic with that of the antennae.

Electrophysiological studies have demonstrated that sensilla within the LPO of female *Pieris rapae* are responsive to natural complex odors such as those of host plants and conspecific animals. Male odor was twice as effective as female odor in stimulating the LPO receptors. No reaction was detected to stimulation by air of different temperature or humidity (Lee et al. 1985). In other studies, antennal sensilla of *Pieris brassicae* showed no (or weak) responses to the odor of the oviposition plant, white cabbage (Behan and Schoonhoven 1978; Den Otter et al. 1980). Lee et al. (1985) therefore suggested that the receptor cells of the LPO are involved in recognition of host or oviposition plants. Because of the strong response to male odor, an interaction between plant and pheromone reception in the female receptor cells could not be excluded. The authors suggested that such an interaction could increase the probability of mating in an environment suitable for oviposition. In our laboratory, however, preliminary behavioral studies have suggested that ablation of the LPO in *M. sexta* does not affect the female's choice of oviposition site, while ablation of the antennae does affect this choice (Harrow, Kovelman, and Hildebrand, in preparation).

The possibility that the LPO plays a role in reproductive behavior is intriguing in light of what is known about the accessory olfactory organ, the vomeronasal organ, in many species of vertebrates. The vomeronasal organ has been shown to be important for reproductive behaviors in both sexes (for example, see Fleming et al. 1979; Marques 1979; Beauchamp et al. 1983; Halpern and Kubie 1983; Hart 1983).

The projection of primary sensory fibers from the LPO to a single antennal-lobe glomerulus suggests that the LPO may have a specific function in *Manduca*. This idea gains support from our observation that the LPO-glomerulus apparently receives little or no sensory input from the antenna (Kent and Hildebrand 1985). The only other example of primary-afferent input to a single, specific glomerulus is the projection of male-specific antennal sensory fibers to the sexually dimorphic macroglomerular complex in the antennal lobe (Camazine and Hildebrand 1979; Schneiderman 1984; Camazine, Harrow, Kent, and Hildebrand, in preparation). Physiological evidence from moths and cockroaches has shown that the macroglomerular complex is a site, or the site, of first-order synaptic processing of olfactory information carried by sensory neurons specialized to detect the female pheromones (Boeckh et al. 1977; Boeckh and Boeckh 1979; Matsumoto and Hildebrand 1981; Christensen and Hildebrand 1984).

Stocker et al. (1983) suggested that individual glomeruli in the antennal lobes of *Drosophila* are functionally specialized, since no evidence for spatial representation of input from flagellar sensilla could be demonstrated. In *Drosophila*, certain antennal glomeruli receive bilateral inputs from

the antennal axons; the remaining glomeruli, therefore, may be the sites of processing of directional information (Stocker et al. 1983). In *Manduca*, the antennal input is strictly unilateral, while the input from the LPO is bilateral. The LPO thus may provide the only non-directional olfactory information to the antennal lobes. We cannot rule out the possibility that input from the LPO provides directional information, however, since we do not know the number or types of synaptic contacts formed by the sensory axons from one LPO in each antennal lobe.

The labial palps of *Drosophila* do not send axons to the antennal-lobe glomeruli and may contain only gustatory receptors. Central projections from taste pegs and bristles on the palps terminate in the SEG (Stocker and Schorderet 1981; Nayak and Singh 1985). In contrast, behavioral studies indicate that the blowfly *Cynomyia* has olfactory receptors on the labial palps (Wigglesworth 1972). Wigglesworth suggested that in the blowfly, antennae function as directional chemoreceptors, while the labial palps may function as non-directional chemoreceptors which, in conjunction with the contact chemoreceptors also on the labial palps, could serve in the reception of combined olfactory and gustatory information during feeding. Central projections of sensory neurons arising from the labial palps of the blowfly have not been described.

Perhaps olfactory receptors on the mouthparts of several species of insects have projections to the SEG and/or tritocerebrum. In adult *Manduca*, gustatory receptors may be confined to the proboscis. Central projections of sensory cells in the proboscis appear to terminate in the SEG and do not pass into the antennal lobe (Camazine, Harrow, Kent, and Hildebrand, in preparation). We also observed arborizations within the SEG after exposing the LPO to cobalt solution, but we cannot be certain that these projections did not arise from mechanoreceptors lying near the LPO or at the joint of the second and third segments of the labial palp. A chordotonal organ is present near the tip of the labial palp in *Pieris* (Kim 1961; Lee et al. 1980).

In conclusion, the labial palps of feeding (*Manduca*) and non-feeding (*Antheraea* and *Bombyx*) species of adult Lepidoptera possess sensilla innervated by neurons that project to a single glomerulus in the ipsilateral and contralateral antennal lobes. Input from the antennae may serve as directional chemoreceptors, helping the animal to locate a host or oviposition plant. In contrast, input from the LPO may provide non-directional information, acting synergistically with input from the antennae or having a special function – as yet undetermined – that is common to both feeding and non-feeding adults of both sexes. Future behavioral and physiological studies should elucidate the function of the LPO and the significance of the LPO-glomerulus in the antennal lobe.

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