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CEPHALIC SENSORY PATHWAYS IN THE CENTRAL NERVOUS SYSTEM OF LARVAL *MANDUCA SEXTA* (LEPIDOPTERA: SPHINGIDAE)

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[Plates 1–3]

CONTENTS

Central projections of neurons innervating sensory structures on the head of larval *Manduca sexta* were traced by using methods of anterograde cobalt-diffusion. Regions of the deutocerebrum and tritocerebrum in the brain receive input from the antenna, labrum, maxilla, labial palps, hypopharynx and other unidentified regions of the buccal cavity. Antennal, maxillary and labial inputs project to the larval antennal centre (LAC) of the deutocerebrum. Stemmatal neurons and a few antennal neurons project into the protocerebrum. The suboesophageal ganglion (SEG) receives input

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from mechanosensory neurons in all parts of the head and its sensory appendages. Some mechanosensory neurons project further to the first thoracic ganglion. In addition to receiving input from chemosensory neurons of the maxilla, the SEG may also receive chemosensory input from epipharyngeal sensilla of the labrum.

INTRODUCTION

To investigate the mechanism of host-plant selection, many studies have compared the feeding behaviour of normal lepidopterous larvae with that of larvae after experimental removal or inactivation of sensory structures believed to have an olfactory or gustatory function (see, for example, Deithier (1937), Waldbauer & Fraenkel (1961), Henson & Dethier (1973) and Ma (1972, 1976)). Some studies have focused on the innervation, morphology or electrophysiology of the sensilla on the antennae and mouthparts (see, for example, Ishikawa et al. (1969), Schoonhoven & Dethier (1966), Hanson (1970), Ma (1972, 1976) and Albert (1980)). Little is known, however, about the central projections of neurons innervating chemosensory sensilla or about the regions of the central nervous system (CNS) involved in integration of chemosensory information and the control of feeding behaviour.

Olfactory sensilla have been found on the antennae and the maxillary palps and galeae of lepidopterous larvae (Morita & Yamashita 1961; Schoonhoven & Dethier 1966; Städler & Hanson 1975; Visser 1983). The olfactory receptors on the antennae allow a caterpillar to orient toward a source of food, but the receptors on the maxillary palps may facilitate feeding (Ishikawa et al. 1969). Gustatory sensilla occur on the palps and galeae of the maxillae, the epipharyngeal surface of the labrum, and possibly the hypopharynx or deeper in the buccal cavity (Dethier 1937; Schoonhoven & Dethier 1966; Ishikawa 1963; Dethier & Kuch 1971; Ma 1972, 1976; Städler & Hanson 1975; De Boer et al. 1977). These sensilla inform the insect of the presence and concentration of nutrients and incitants and inhibitors of feeding (Visser 1983). Mechanosensory sensilla are found on all mouthparts as well as on the antennae. In addition to coordinating the movement of the mouthparts and the manipulation of food, mechanosensory inputs give the animal information about the proximity of food and its texture or consistency (Hanson 1970; Städler & Hanson 1975).

The neural centres for primary integration and processing of sensory information from receptors on particular appendages are presumably located in the neuromeres of the CNS corresponding to the body segments carrying the appendages (Snodgrass 1960). Indeed, tracing the nerve innervating a particular segmental appendage has been one way of identifying the corresponding neuromere after fusion and condensation of CNS elements during development to form complex ganglia. The antennal nerve projects to the deutocerebrum, the labral nerve to the tritocerebrum, and the labial, maxillary, and mandibular nerves to the corresponding neuromeres in the suboesophageal ganglion (SEG). Central projections of antennal axons have been examined in several species of insects (bees (Pareto 1972; Suzuki 1975), cockroaches (Boeckh et al. 1970), flies (Stocker et al. 1983) and moths (Camazine & Hildebrand 1979; Hildebrand et al. 1980)). Input to the brain and SEG from the other gustatory and olfactory organs has not been as widely studied. In Drosophila, central projections of neurons innervating sensilla on the mouthparts have been found in the SEG and tritocerebrum (Stocker & Shorderet 1981). In locusts, neurons innervating wind-sensitive hairs on the head and those innervating the hairplate and campaniform sensilla on the antenna and

mouthparts project to regions of the deutocerebrum, tritocerebrum, SEG and thoracic ganglia (Tyrer *et al.* 1979; Bräunig *et al.* 1983).

As part of our continuing study of postembryonic development of sensory pathways in *Manduca sexta* and in preparation for studies of central control of feeding behaviour, we have traced the projections of mechanosensory and chemosensory axons from sensilla of the antenna and mouthparts into the CNS of larval *Manduca*. Axons of neurons innervating sensilla on particular segmental appendages project to, and ramify within, the appropriate neuromeres in the brain and SEG. In most cases the primary-afferent fibres also project further into other neuromeres, where their terminal fields overlap with inputs from other sensory organs.

Materials and methods

Larval *Manduca sexta* (Lepidoptera: Sphingidae) were reared on artificial diet (Bell & 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 & Hildebrand 1976). In preparation for experimental manipulation, fifth-instar larvae were anaesthetized by chilling on ice.

To fill all axons in a particular peripheral nerve with cobalt solution, we excised as much of the nerve as possible along with the brain, SEG and first thoracic (prothoracic) ganglion (TG1) and placed the tissue in a Petri dish coated with Sylgard (Dow Corning). The nerve was draped over a petroleum-jelly bridge into a pool of 150 mM or 300 mM cobaltous nitrate or a dilute solution of a cobaltous-lysine complex (Lazar 1978), and the ganglia (brain, SEG, TG1) were immersed in a drop of Grace's insect culture medium (GIBCO) (Grace 1962) or *Manduca* saline (Cherbas 1973). Preparations were incubated at 4 °C for 8–24 h. To fill neurons innervating an individual sensillum, we pinned the head on a slab of Sylgard, cut the tip of the sensillum, and placed a micropipette filled with aqueous cobaltous nitrate (150 mM–1 M) or cobaltous-lysine complex over the sensillum. To fill axons from groups of sensilla or a very small sensillum, we cut the sensilla or the tip of the appendage carrying them, constructed a petroleum-jelly well around the appendage, and applied one of the cobalt solutions.

After filling cells with cobalt, we precipitated it with aqueous ammonium sulphide, sodium sulphide or hydrogen sulphide. The ganglia were fixed in Carnoy's solution (Humason 1972) or ammoniacal alcohol (N. J. Strausfeld, personal communication) for wholemounts, or in alcoholic Bouin's solution (Humason 1972) in preparation for sectioning. Ganglia or 8 μm paraffin sections were subsequently processed according to modifications of Timm's silver intensification method (Bacon & Altman 1977; Tyrer & Bell 1974). Intensified wholemounts were embedded in Epon (Cargille) and sectioned at 3–6 μm with a Sorvall MT2B Ultramicrotome equipped with a glass knife.

Each central projection described below was observed a minimum of three times in different preparations. In many cases, a particular projection profile was observed in over 20 preparations.

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RESULTS

The antennal pathway

As described for a variety of Lepidoptera by Dethier (1941), the larval antenna consists of three segments (see figure 1, plate 1; figure 2, A). The innervation of the antenna in larval *Manduca* has been described (Schoonhoven & Dethier 1966). The first or basal segment bears singly innervated *sensilla campaniformia* and a 'free' nerve ending (a single neuron innervating a site that lacks visible cuticular modification (Schoonhoven & Dethier 1966)) (neither shown in figure 2, A). The second segment carries two singly innervated tactile hairs (Ch1 and Ch2), three multiply innervated *sensilla basiconica* (two large, K1 and K2, and one small, k1), a
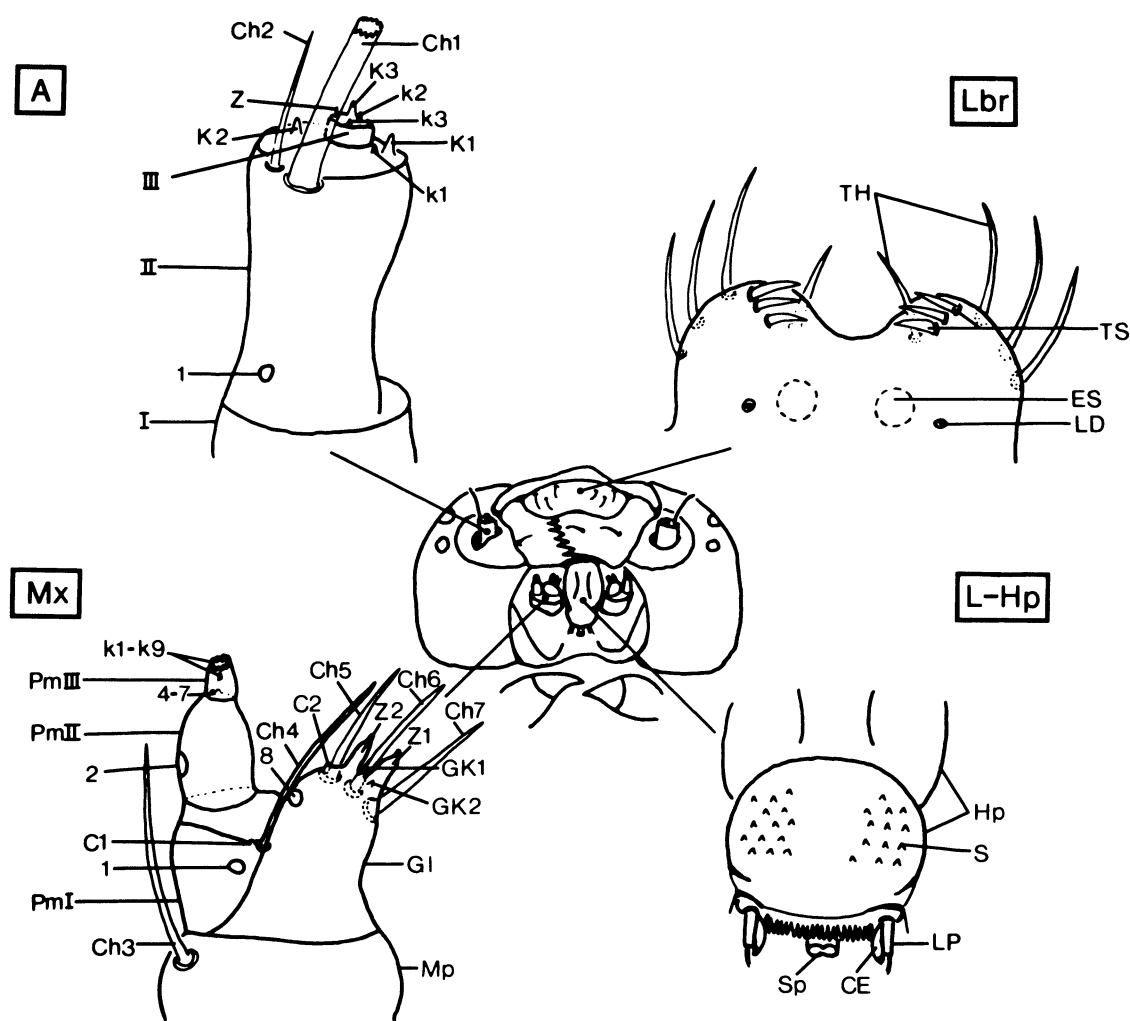

FIGURE 2. Diagram of sensory appendages on the larval head. (A) Segments I, II and III of the antenna: 1, *sensillum capaniformium*; Ch1, Ch2, tactile hairs; k1–k3, small *sensilla basiconica*; K1–K3, large *sensilla basiconica*; Z, *sensillum styloconicum*. (Lbr) Epipharyngeal surface of the labrum: ES, region of epipharyngeal sensilla; LD, lateral domes; TH, tactile hairs on anterior surface of labrum; TS, tactile setae. (Mx) Maxilla: Mp, palpiger; Gl, galea; PmI, PmII, PmIII, first, second, and third segments of the maxillary palp; C1–C2 and 1–8, *sensilla campaniformia*; Ch3–Ch7, tactile hairs; GK1–GK2, *sensilla basiconica* of the galea; k1–k9, *sensilla basiconica* of the palp; Z1–Z2, *sensilla styloconica*. (L-Hp) Labial-hypopharyngeal complex: Hp, hypopharynx; LP, labial palp; CE, cuticular extension of labial palp; S, spines; Sp, spinneret. (A) and (Mx) after Schoonhoven & Dethier (1966); larval head after Visser (1983).

FIGURE 1. Scanning electron micrograph of the ventral surface of the head of fifth-instar larval *Manduca sexta*. A, antenna; Hp, hypopharynx; Lbr, labrum; L, postmentum of labium; LP, labial palp; Mx, maxilla; Md, mandible; MdTH, tactile hair on mandible; MS, maxillary stipes; Md, mandible; MdTH, tactile hair on mandible; Sp, spinneret; St, stemmata; TH, tactile hair on head. Scale bar: 500 μm.

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Kent & Hildebrand, plate 2
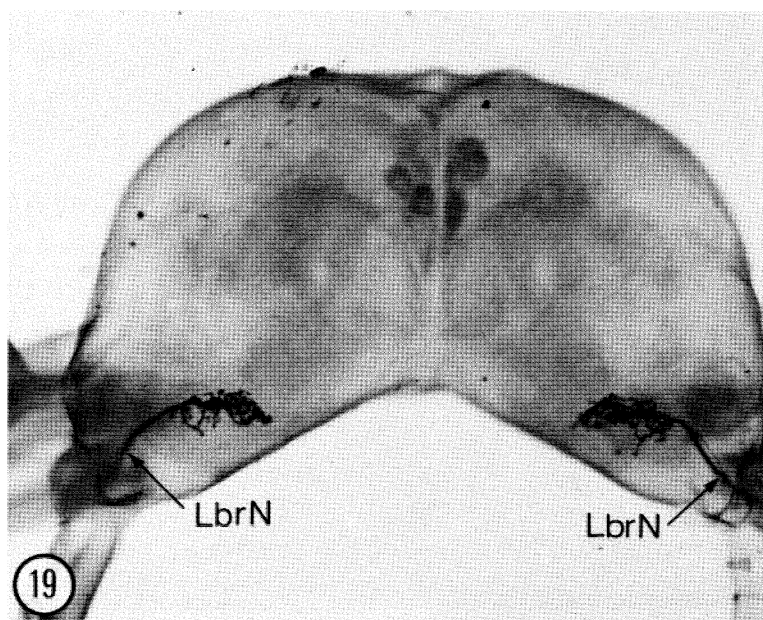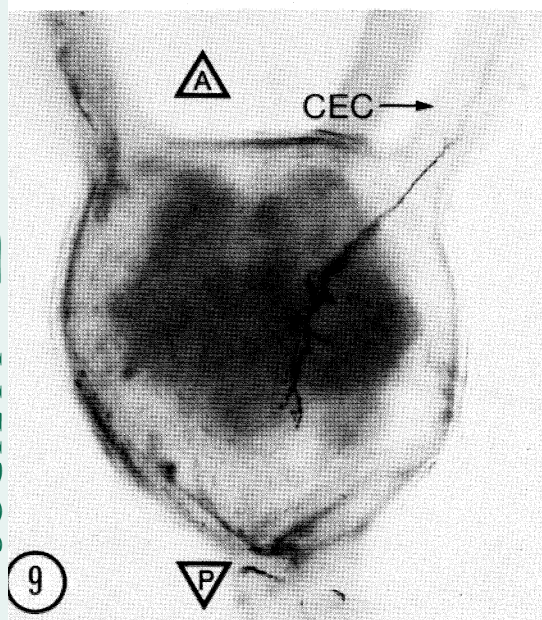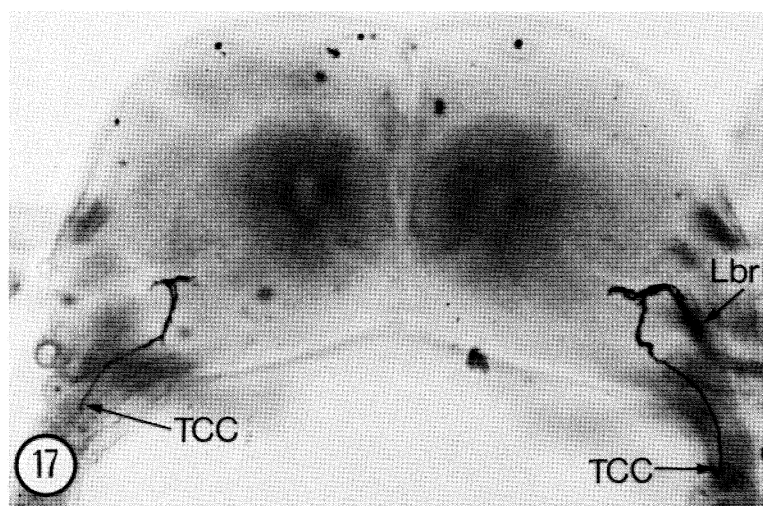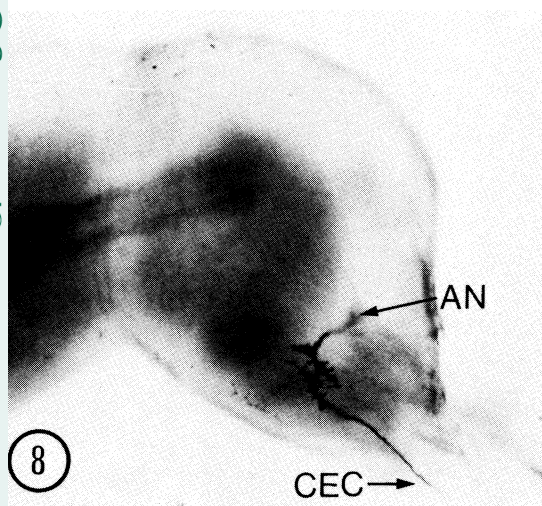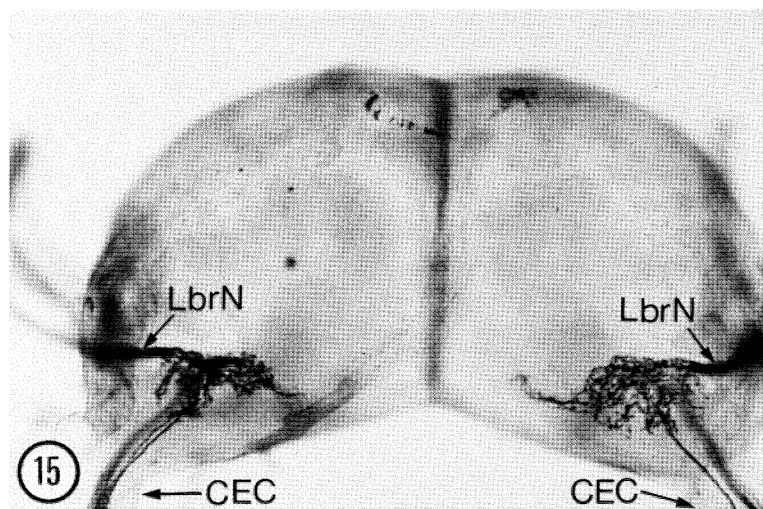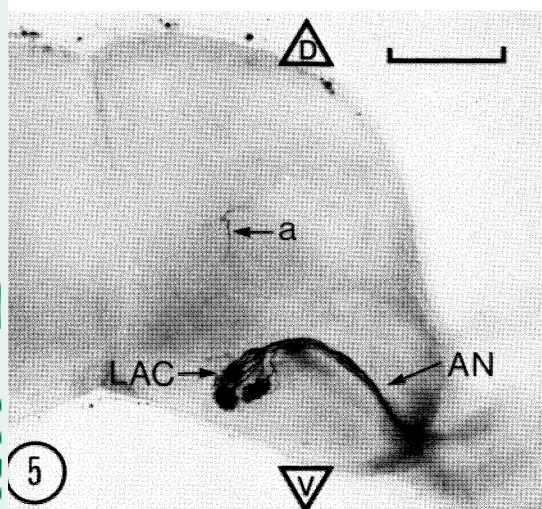
FIGURES 5, 8, 9, 15, 17 AND 19. For description see opposite.

DESCRIPTION OF PLATE 2

FIGURE 5. Anterior view of a wholemount of the brain showing antennal axons entering via the antennal nerve (AN) and forming tufted arborizations in the LAC. Part of one of the fibres that passes beyond the LAC into the protocerebrum is in the same plane of focus (a). D, dorsal; V, ventral. All subsequent wholemounts of the brain have a similar orientation. Scale bar: 100 μm.

FIGURE 8. Anterior view of a wholemount of the brain showing the fibre from Ch1 on the antenna. The fibre passes through the brain to the CEC.

FIGURE 9. Ventral view of a wholemount of the SEG showing additional arborizations of the fibre in figure 8. A, anterior; P, posterior.

FIGURE 15. Anterior view of a wholemount of the brain drawn in figure 14.

FIGURE 17. Anterior view of a wholemount of the brain drawn in figure 16.

FIGURE 19. Anterior view of a wholemount of the brain showing the staining pattern observed after the entire buccal cavity had been exposed to cobalt solution. Cell bodies near the dorsal midline of the brain turned dark after silver intensification of this preparation. These cells are part of the group of medial neurosecretory cells (Nijhout 1975; Buys & Gibbs 1981). The reason for their staining is not known. Transneuronal staining of these neurons cannot be ruled out although none of their processes stained.
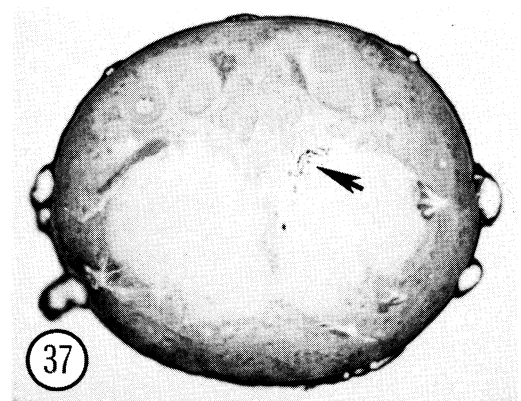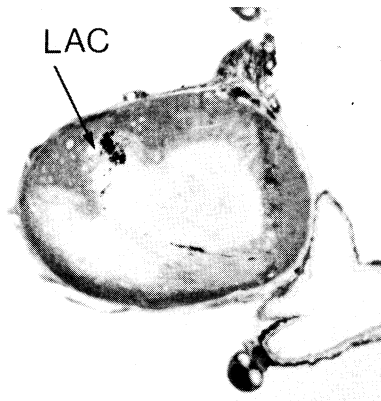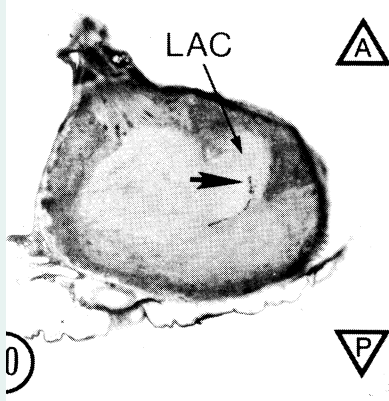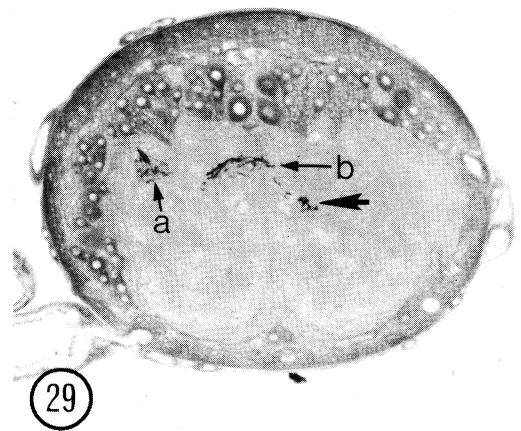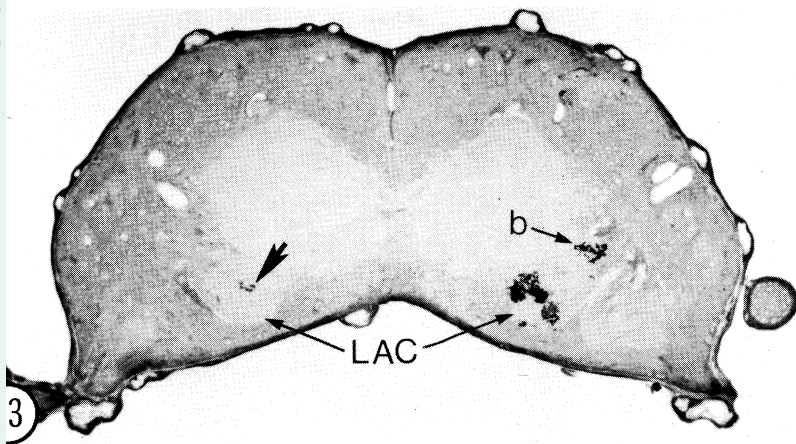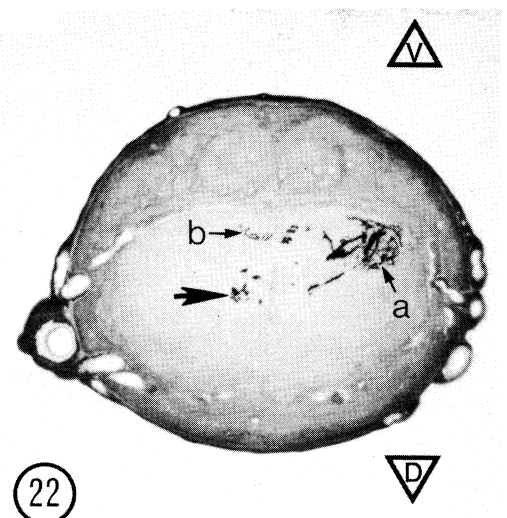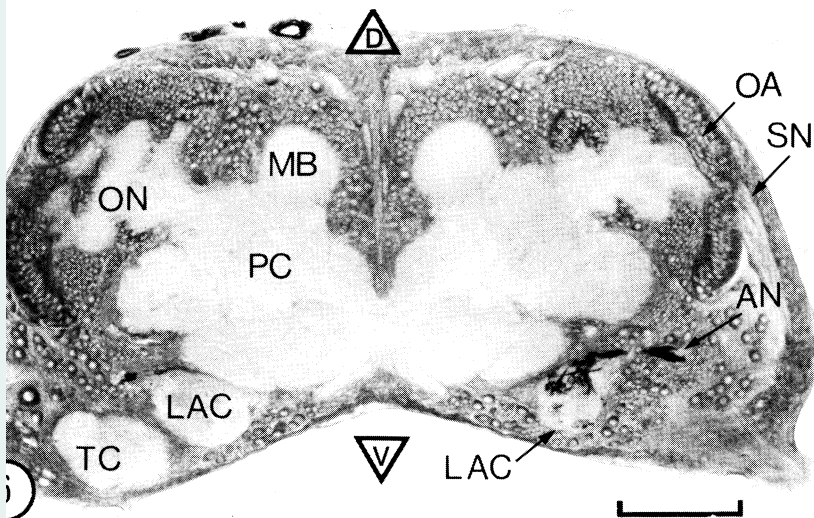
FIGURES 6, 22, 23, 29, 30 AND 37. For description see opposite.

campaniform sensillum (1), and two 'free' nerve endings. The third segment bears one large basiconic sensillum (K3), two small basiconic sensilla (k2, k3), and one *sensillum styloconicum* (Z), all multiply innervated.

For selective staining of neurons innervating certain groups of sensilla on the antenna, we applied cobalt solution after cutting the antenna at the level of the first, second, or third segment. Many fine fibres were stained that run in the antennal nerve (AN in figure 3) and project medially to a region of neuropile near the anteroventral surface of the brain. These fibres densely innervate a knot of nodular neuropile, the larval antennal centre (LAC), within the deutocerebrum (figure 4, B; figure 5, plate 2; figure 6, plate 3). Although we have not attempted to count the fibres, it appears that a majority of antennal axons, probably representing the larger chemosensory fraction of antennal neurons, project to the LAC. Emerging from the LAC, one or two fine fibres (a in figure 4, B, B(sag); figure 5) project further medially and then dorsally and ramify diffusely in uncharacterized regions of the ipsilateral protocerebrum.

Other fibres, some of which appear to be of large diameter, enter the brain via the antennal nerve and project directly posteriorly, sending out stubby processes (b in figure 4, B, B(sag)) as the fibres proceed toward the ipsilateral circumoesophageal connective (CEC). Some of these axons have longer medial processes that branch diffusely in a region posterior and ventral to the LAC (c in figure 4, B). In addition one of these processes loops back toward the LAC, forming a small tuft that abuts the posterior side of the LAC (d in figure 4, B). After travelling through the CEC to the SEG, the large fibres enter dorsally and advance toward the ventral side of the SEG as they project posteriorly to a central core of neuropile at the medial edge of the ipsilateral hemiganglion (figure 4, SEG, SEG(sag); large arrow in figures 22 and 29, plate 3). In this central neuropile the fibres send out short branches. In some preparations, we observed that a single fibre projects beyond the SEG and terminates in TG1 (figure 4, TG1).

DESCRIPTION OF PLATE 3

FIGURE 6. An 8 μm oblique paraffin section through the brain. Antennal axons (AN) were stained by exposing the cut end of the antenna to cobalt solution. Sections were intensified with silver. Tufted arborizations are apparent in the LAC. Other regions of neuropile apparent include tritocerebrum (TC), protocerebrum (PC), mushroom bodies (MB) and optic neuropile (ON). Larval stemmatal axons (SN) and optic anlage for the adult optic lobe (OA) are visible in this plane of section. D, dorsal; V, ventral. Scale bar: 100 μm.

FIGURE 22. A 6 μm frontal plastic section through the SEG at the level of the maxillary neuromere, showing fibres that stained after the maxillary palp and galea (right) and antenna (large arrow, left) had been cut and exposed to cobalt solution. a, Rounded array of maxillary arborizations; b, maxillary fibres that fork at the midline and cross to the contralateral side. D, dorsal; V, ventral.

FIGURE 23. A 6 μm frontal plastic section through the brain, showing cobalt staining in the LAC after staining antennal axons (right) and maxillary axons (large arrow, left). b, Tract of antennal fibres that run to the CEC and SEG. Dorsal–ventral orientation as in figure 6.

FIGURE 29. An 8 μm frontal paraffin section through the SEG at the level of the labial neuromere. Cobalt staining shows initial region of arborization of labial axons (a, left) and fibres that run medially and cross the midline (b). On the right (large arrow) are stained antennal fibres demonstrating relative positions of labial sensory fibres and the core of neuropile in which antennal axons travel. Dorsal–ventral orientation as in figure 22.

FIGURE 30. Two 6 μm horizontal plastic sections through the ventral region of the larval brain. Right, cobalt-stained arborizations of antennal fibres in the LAC; Left, projection of labial fibre (large arrow) to posterior edge of LAC. A, anterior; P, posterior.

FIGURE 37. A 6 μm frontal plastic section at the level of the mandibular neuromere. Right side shows cobalt staining (arrow) that resulted after mandibular teeth had been cut and bathed in cobalt solution. Dorsal–ventral orientation as in figure 22.
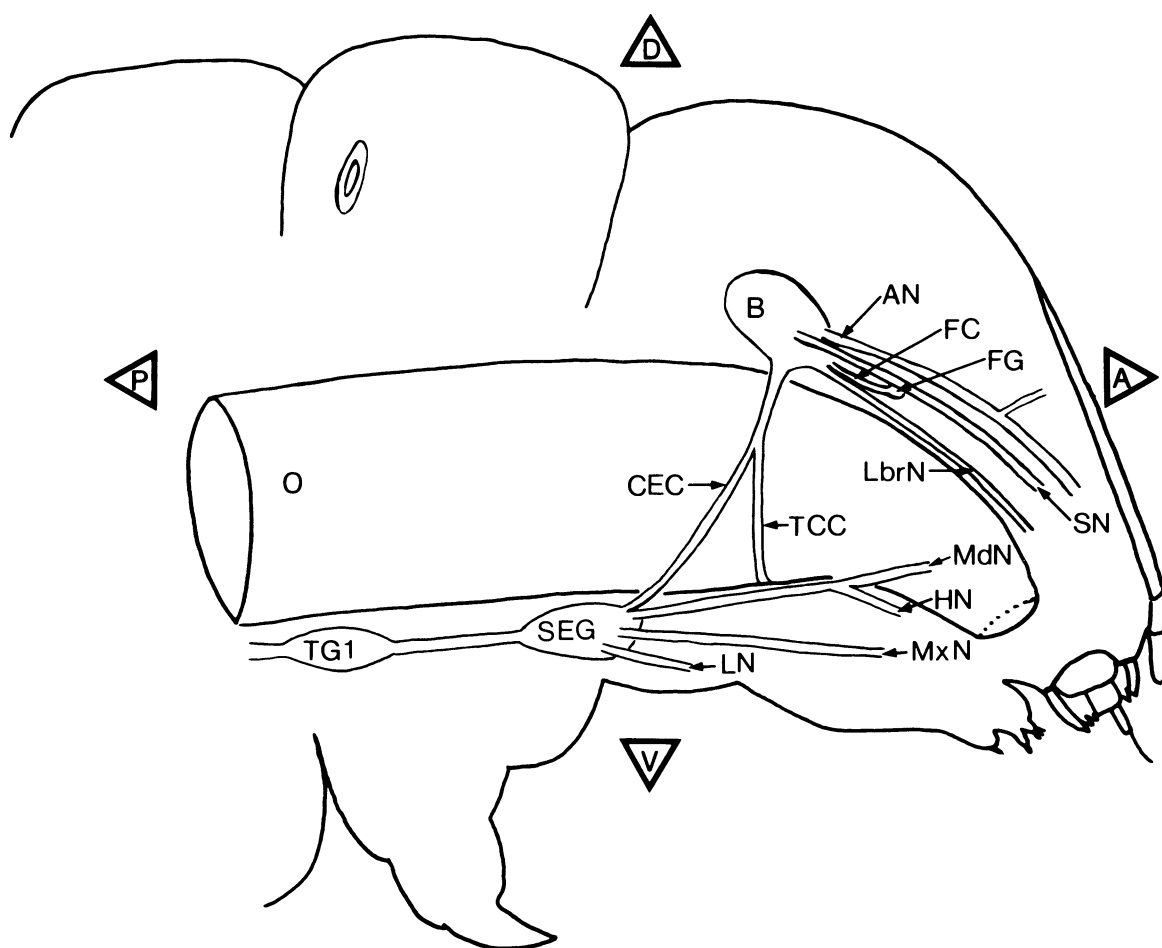
FIGURE 3. Diagram of the larval brain (B), suboesophageal ganglion (SEG), first thoracic ganglion (TG1), and relevant nerves as they are oriented within the head. AN, antennal nerve; CEC, circumoesophageal connective; O, oesophagus; FC, frontal connective; FG, frontal ganglion; HN, hypopharyngeal nerve; LN, labial nerve; LbrN, labral nerve; MdN, mandibular nerve; MxN, maxillary nerve; SN, stemmatal nerve; TCC, tritocerebral commissure. Anterior (A), posterior (P), dorsal (D) and ventral (V) are indicated in triangles in this and other figures.

Selective staining of the neuron innvervating the large tactile hair (Ch1) on the second segment of the antenna revealed that its axon is one of the large-diameter fibres that terminate in the SEG (a in figure 7; figures 8 and 9, plate 2). This group of fibres also includes the axon of the neuron innervating Ch2 and probably the axons associated with the campaniform sensilla and 'free' nerve endings in the antenna. A similar projection is characteristic of the neuron innervating the tactile hair on the head cuticle just dorsal to the antenna socket (TH in figure 1) (figure 10).

We do not know the origin of the longer medial processes that appear to arise from this tract of heavy fibres and project to a region posteroventral to the LAC. When the third antennal segment alone had been cut, the staining pattern still included fibres that had medial processes in the region posterior to the LAC and axons projecting to the SEG (b and c in figure 11). This finding is consistent with the possibility that these medial processes arise from chemosensory fibres, which may project further to the SEG. It is also possible that certain sensilla in the third
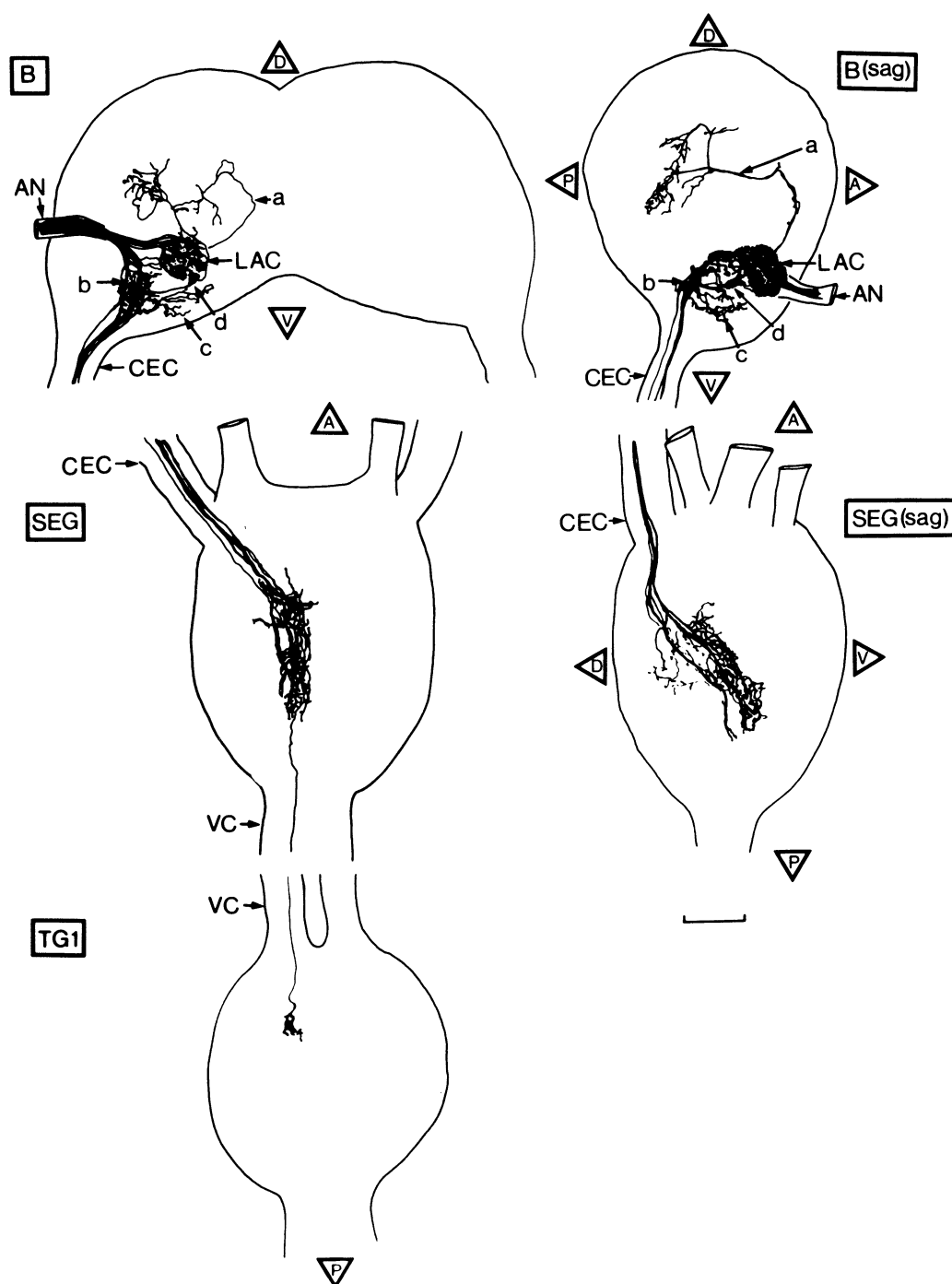
FIGURE 4. *Camera lucida* drawings of central projections of antennal axons that stained after cobalt solution had been applied to the cut end of the first antennal segment. An anterior view of the brain (B) and a sagittal view of the brain (B(sag)) show many fibres that pass through the antennal nerve AN to the larval antennal centre LAC. a, Fine fibres that project beyond the LAC into the protocerebrum; b, axons that project into the CEC and SEG; c, medial arborizations; d, tufted process abutting the posterior side of the LAC. A ventral view of the SEG (SEG) and a sagittal view of the SEG (SEG(sag)) show further projections of fibres from the brain. VC, Ventral connective. A ventral view of TG1 (TG1) shows a single fibre that was stained in a few preparations. Scale bar: 100 μm.
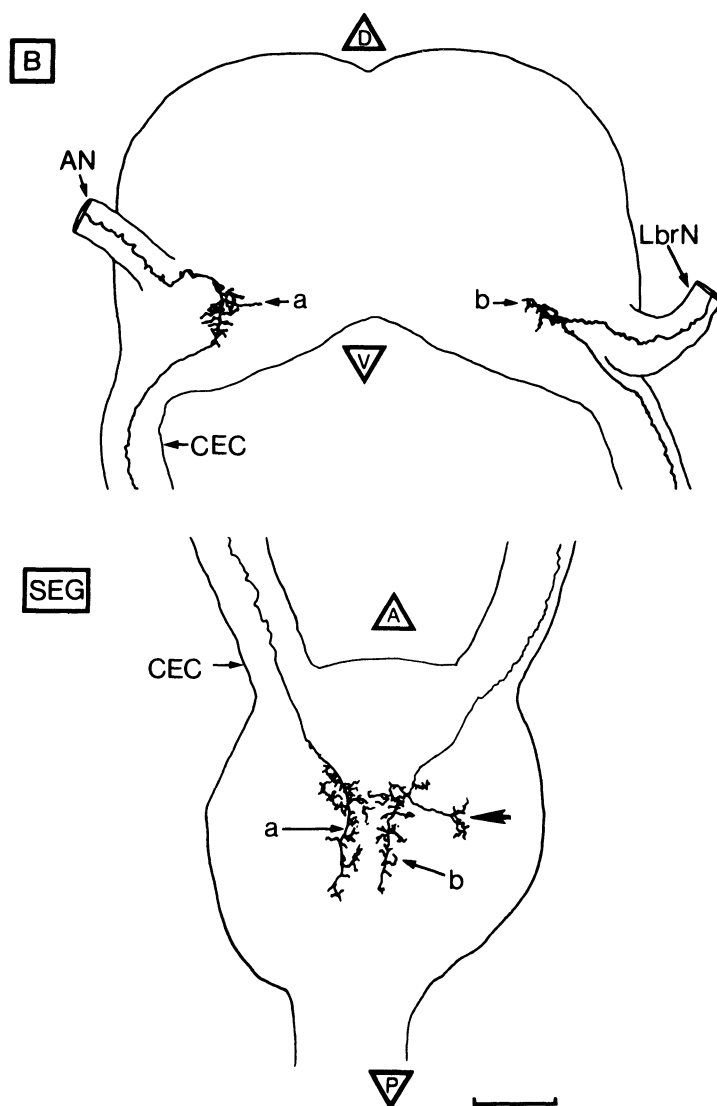
FIGURE 7. *Camera lucida* drawings of an anterior view of the brain and a ventral view of the SEG showing single fibres that stained after cobalt solution had been applied to cut tactile hairs of the head. a, Fibre arising from the large tactile hair (Ch1) on the antenna; b, fibre arising from tactile hair on the anterior surface of the labrum. In the SEG, the large arrow indicates the branch that forks in more ventral neuropile. Scale bar: 100 μm.

antennal segment are innervated by mechanosensory neurons. For example, the styloconic sensillum (Z in figure 2, A) of the third segment might be innervated by at least one mechanosensory cell, as are the *sensilla styloconica* of the maxillary galea (Schoonhoven & Dethier 1966). Schoonhoven (1967) suggested that the antennal styloconic sensillum may be innervated by cold-sensitive receptors. Finally, we cannot rule out the possibility that cobalt may have leaked from the third segment and stained mechanosensory cells in the second segment.

To confirm that the fibres projecting to the LAC originated in the antenna and were not stained as a result of leakage of cobalt to non-antennal axons running in the antennal nerve, we excised one antenna during one or two preceding larval instars to cause degeneration of the antennal sensory neurons. For cobalt filling on the deantennated side, we cut the
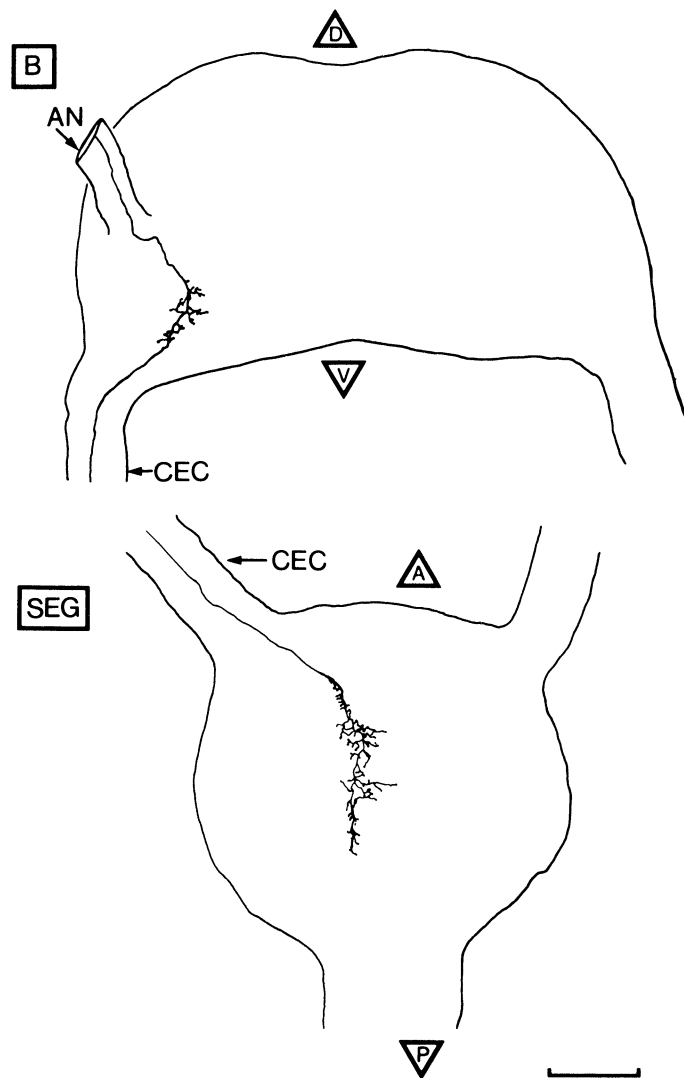
FIGURE 10. *Camera lucida* drawings of an anterior view of the brain and ventral view of the SEG showing a fibre arising from a tactile hair TH (see figure 1) on the head cuticle dorsal to the antenna. Scale bar: 100 μm.

membranous centre of the empty antennal socket or a small stump of cuticle of the first segment that remained in some cases. We cut the contralateral, normal antenna at the same level and constructed petroleum-jelly wells filled with cobalt solution around both antennal sockets. The resulting pattern of staining revealed that the entire LAC projection was absent on the deantennated side (figure 12). In cases in which a stump of the first antennal segment remained, one or two coarse fibres were stained. The profiles of these fibres resembled those of the neurons innervating Ch1 and tactile hairs on the head cuticle (compare figures 7, 10 and 12).

In addition to filling antennal axons from the cut end of the antenna or the empty antennal socket, we stained axons in the antennal nerve by dipping cut antennal nerves of normal and deantennated animals in cobalt solutions. This ensured that the lack of staining on the deantennated side of the brain was not the consequence of insufficient penetration of the cobalt
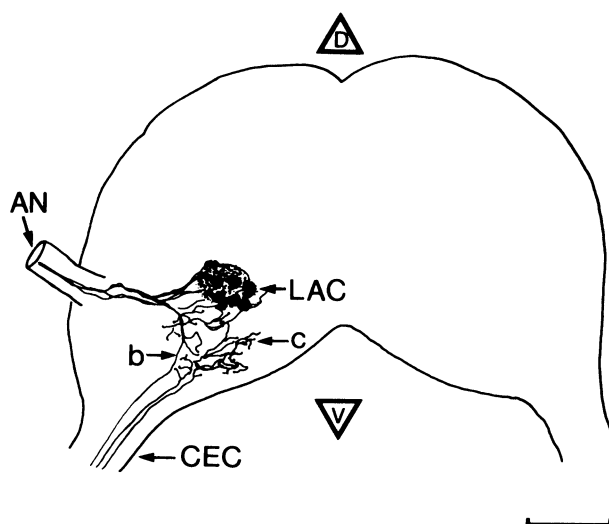
FIGURE 11. A *camera lucida* drawing of an anterior view of the brain showing fibres that stained after cobalt solution had been applied to the cut third segment of the antenna. b, Fibres that project on to the CEC and SEG; c, medial arborizations. Scale bar: 100 μm.

solution. Staining antennal nerves in this way also revealed that the projection to the LAC was absent on the deantennated side (figure 13, B), although many additional fibres stained on both sides. Several of the coarse fibres remained (b in figure 13, B, SEG) and probably came from neurons innervating tactile hairs on the head cuticle and from campaniform sensilla in the first segment of the antenna, which had not been completely excised. Some of these fibres projected further to TG1 (figure 13, TG1). In some cases one of the fine fibres that usually emerge from the LAC was still present. That process emerged from the antennal nerve and projected medially across the region (LAC) that would have received antennal axons in a normal preparation (a in figure 13, B).

Although the fine fibres that emerge from the vicinity of the LAC and project into the protocerebrum are reminiscent of the axons of antennal-lobe output neurons that pass to the calyces of the mushroom bodies and the lateral lobe of the protocerebrum through the tractus olfactorioglobularis in adult *Manduca* (Montague *et al.* 1983; Hildebrand & Montague 1985), we have not seen stained cell bodies in any preparations in which antennal axons were stained by applying cobalt solution to the cut end of the antenna. It is therefore unlikely that the fine fibres belong to similar relay interneurons. Because we sometimes saw one of these fine fibres in deantennated preparations in which the remaining antennal nerve had been filled with cobalt, at least one of these fibres may not be a primary antennal axon. We cannot rule out the possibility that, in these deantennated preparations, the fine processes resemble but are distinct from the fine fibres that stained after we applied cobalt solution to the cut end of the antenna. It is also possible that these fine fibres come from receptor cells that lie at the base of the antenna and escaped excision in some deantennated animals.

Although staining antennal axons by filling from the cut end of the antenna revealed no central cell bodies, filling cut antennal nerves with cobalt solution at the level of branches to the antennal muscles led to staining of two groups of neuronal cell bodies on both the normal and the deantennated sides of the brain. This staining revealed two or three large cell bodies anterior to the LAC neuropile (e in figure 13, B) and several smaller cell bodies (f in figure
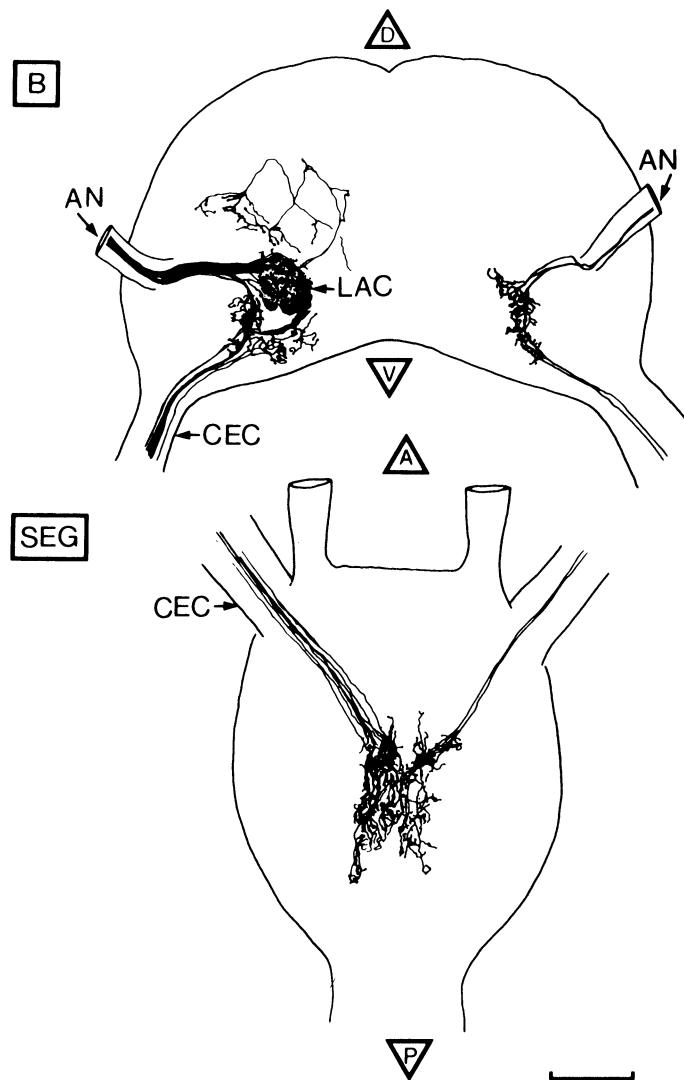
FIGURE 12. *Camera lucida* drawings of the brain (anterior view) and SEG (ventral view) comparing central projections of fibres that stained on normal (left) and deantennated (right) sides. Staining was achieved by applying cobalt to the cut end of the antenna and empty antennal socket. Scale bar: 100 μm.

13, B) lateral to the tract of heavier sensory fibres that project posteriorly toward the CEC. The neurites from the larger cell bodies appear to have arborizations in the region of neuropile that receives medial branches of the heavier sensory fibres (b and c in figure 13, B). An additional region of arborization that extends into the tritocerebrum is apparent lateral to this tract of fibres (g in figure 13, B). Some, if not all, of these stained cell bodies are motorneurons that innervate the antennal muscles.

The labral pathway

Several sensilla are distributed over the outer surface of the labrum (Lbr in figure 1 and figure 2, Lbr). These sensory hairs are undoubtedly tactile, but to our knowledge they have not been studied electrophysiologically in *Manduca*. The inner or epipharyngeal surface of the
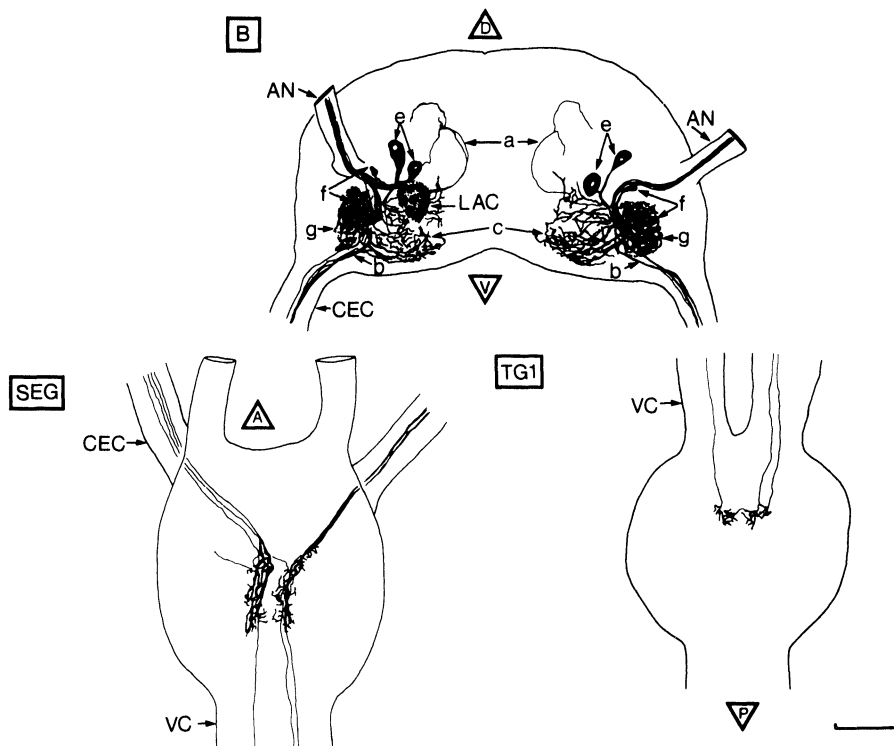
FIGURE 13. *Camera lucida* drawings of the brain (anterior view), SEG (ventral view), and TG1 (ventral view) comparing central projections of fibres that stained on normal (left) and deantennated (right) sides. Staining was achieved by dipping the antennal nerves in pools of cobalt solution. a, Fine fibres that project beyond the LAC into the protocerebrum; b, axons that project into the CEC and SEG; c, medial arborizations; e, large cell bodies; f, small cell bodies; g, lateral arborizations. Scale bar: 100 μm.

labrum bears three flattened tactile setae on the ventrolateral margin of each lobe (TS in figure 2, Lbr). A campaniform sensillum is present on each side of the epipharynx near an epipharyngeal sensillum, a dome-shaped structure with a small papilla on top. Both structures are too small to be seen with the dissecting microscope. Their location on the epipharynx is indicated by a broken circle (ES) in figure 2, Lbr. In *Manduca* each epipharyngeal sensillum is a contact chemoreceptor innervated by three bipolar neurons (Ma 1972; De Boer *et al.* 1977). Lateral to the region of the epipharyngeal sensillum sits a larger dome-shaped structure (LD in figure 2, Lbr) of undetermined innervation and function (G. De Boer, personal communication).

To stain neurons innervating epipharyngeal sensilla, regions medial to the lateral domes were pierced or scraped with the tip of a micropipette and bathed in cobalt solution. Stained by such procedures were several axons that enter the brain via the labral nerve (LbrN in figure 3). Many medially projecting branches stain in the tritocerebrum, including a few longer medial processes ventral to the LAC (figure 14, B; figure 15, plate 2). Some fibres pass through the CEC to the SEG, where they enter the ganglion dorsally and travel in a ventral direction to an ipsilateral region of central neuropile near the midline (figure 14, SEG). Processes extend anteriorly and posteriorly in this central neuropile and ramify along their length. At the level of the maxillary neuromere, a long branch arises from one of the posteriorly directed processes and projects to a more ventral region of neuropile, where it forks (large arrow in figure 14, SEG). Often, in these preparations, however, more than three fibres were stained in the labral nerve and in the CEC. We probably also stained the neurons of the campaniform sensilla in

this region. The cobalt solution may have leaked into axons from other tactile sensilla as well. Preparations stained by filling tactile hairs on the labrum do demonstrate a projection to the SEG with branching profiles similar to that observed after applying cobalt solution to the region of the epipharyngeal sensilla (b in figure 7).
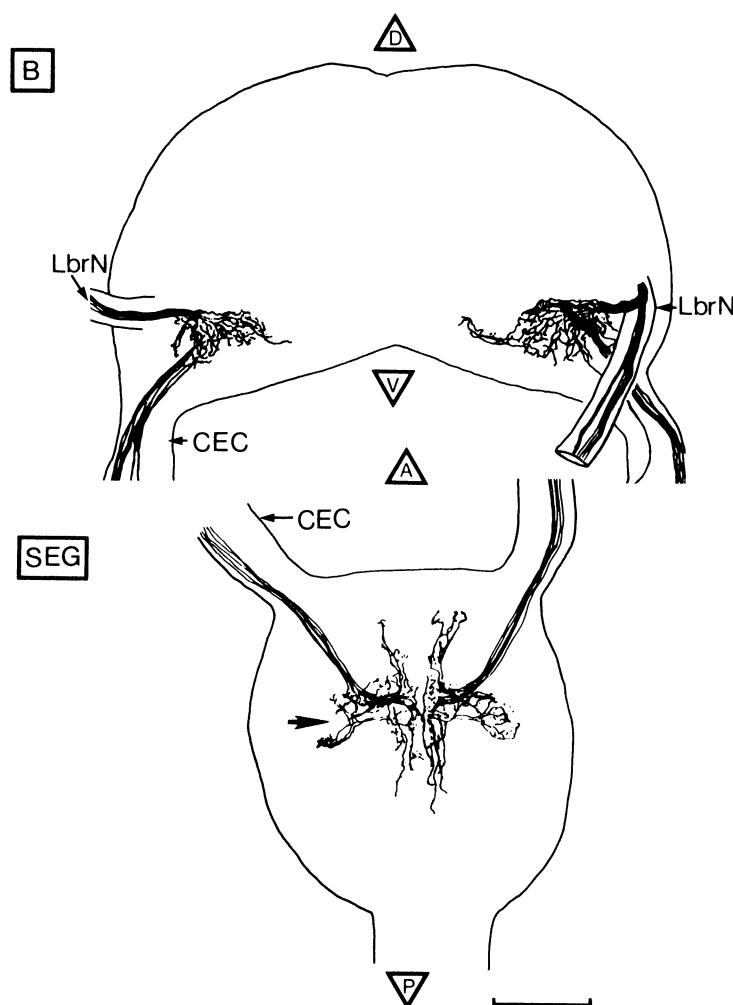

FIGURE 14. *Camera lucida* drawings showing fibres that stained after the ES region had been pierced with a micropipette and exposed to cobalt solution. An anterior view of the brain shows arborizations in the tritocerebrum. A ventral view of the SEG shows terminal arborizations of some fibres that project beyond the brain. Large arrow indicates branches that fork in more ventral neuropile. Scale bar: 100 μm.

To stain neurons innervating a lateral dome, we pierced it with the tip of a glass micropipette before bathing the dome with cobalt solution, or we pierced the dome with a micropipette filled with cobalt solution and left the micropipette in place for 12–24 h. One to three axons enter the brain via the labral nerve and form short, medially directed arborizations in a region of the tritocerebrum lateral to the LAC (figure 16; figure 17, plate 2). At least two of these fibres pass through the tritocerebral commissure (TCC in figure 3) to the contralateral side of the brain, where they have terminal arborizations in the corresponding region of contralateral tritocerebral neuropile.

In some experiments, in which nothing was cut, scraped or pierced and cobalt solution was
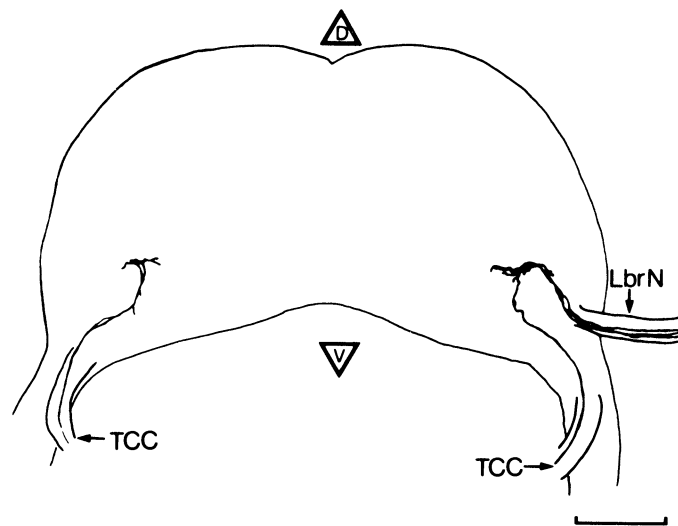
FIGURE 16. *Camera lucida* drawing of an anterior view of the brain showing fibres that stained after the lateral domes had been pierced and exposed to cobalt solution. Fibres enter the brain via LbrN and cross to contralateral side of the brain via the TCC. In this preparation the TCC was cut at points where it joins the CEC after staining was completed. Scale bar: 100 μm.

applied in the region of the epipharynx, hypopharynx or buccal cavity, one to three fibres in the labral nerve were stained. These axons projected to the tritocerebrum, where they had arborizations, but not beyond the tritocerebrum into the CEC or TCC. These fibres did not exhibit the longer, medially projecting branches observed after the region of epipharyngeal sensilla was pierced or scraped (figure 18 *a*; figure 19, plate 2). Fibres with similar projections were also stained in preparations of animals whose labrum had been covered with petroleum jelly or excised in an earlier instar (figure 18 *b*), suggesting that at least some of these fibres arise from sensilla on the hypopharynx or elsewhere in the buccal cavity. Ma (1976) suggested the presence of gustatory cells in the buccal cavity of *Pieris* and *Spodoptera* on the basis of observations that animals lacking the antennae, maxillae, and labrum still reject certain non-host plants. Furthermore, animals whose maxillae and labrum have been removed eat more if sugar is included in the diet (Ma 1976).

Neurons innervating individual tactile hairs on the outer surface of the labrum and clypeus (a triangular region of cuticle dorsal to the labrum on the anterior surface of the head) send axons into the brain via the labral nerve. These fibres give out a few short medial branches as they run posteriorly to the CEC (figures 7, B and 20, B). In the SEG these axons have different projection patterns depending upon the location of the tactile hairs at which they originate: for example, a fibre from a tactile hair on the anterior surface of the labrum enters the SEG dorsally and travels ventrally to central neuropile. A posteriorly directed process ramifies as it travels in this central neuropile and extends one long lateral process that forks in more ventral neuropile at the level of the maxillary neuromere (b in figure 7, SEG). Contrasting with this example, a fibre from a tactile hair on the clypeus enters the SEG dorsally and travels to more ventral neuropile, turning to run posteriorly through the neuropile of the SEG and putting out stubby processes. Such axons project to TG1, where they have terminal arborizations at the ventral midline of the ipsilateral anterior quadrant (figure 20, TG1). Similar projection profiles characterize sensory cells innervating hairs that lie lateral to the
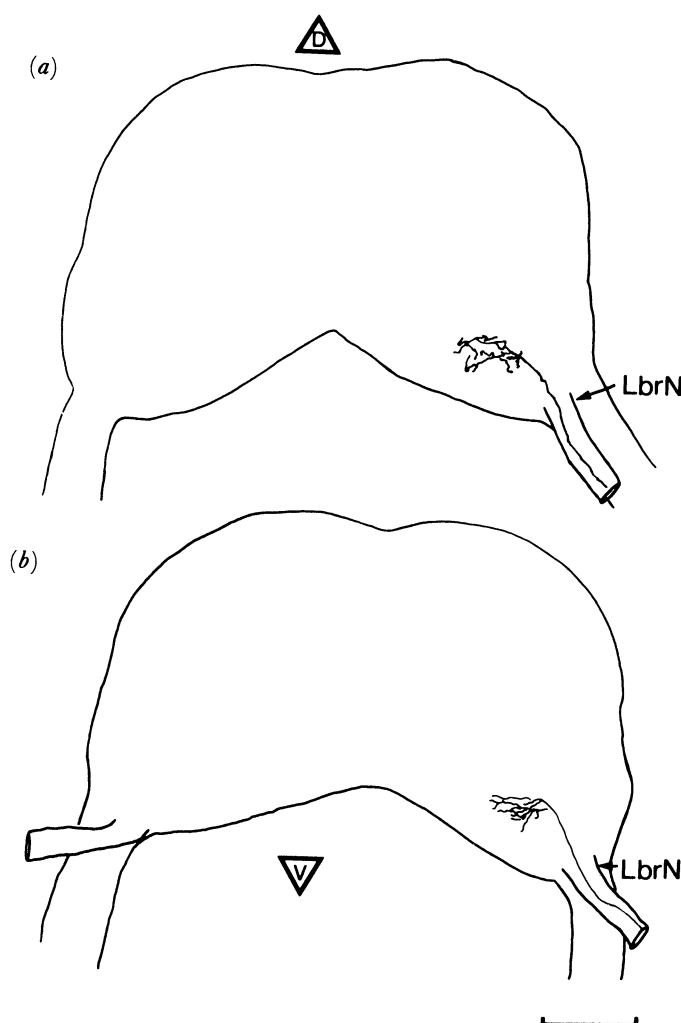
FIGURE 18. *Camera lucida* drawings of anterior views (*a*, *b*) of brains showing single fibres that stained after the buccal cavity and hypopharynx had been exposed to cobalt solution. In (*b*) the labrum had been excised in an earlier instar. Scale bar. 100 µm.

clypeal triangle on the head cuticle. The fibres from these cells, however, enter the brain via the *nervus corporis cardiaci* III (Nijhout 1975; not shown here) and a branch of the antennal nerve.

The maxillary pathway

The maxilla (Mx in figure 1; figure 2, Mx), as described by Schoonhoven & Dethier (1966), comprises two appendages, the palpus (or palp) and the galea, which arise from the basal segment or palpiger (Mp in figure 2, Mx). The base of the palpiger is connected to a sac-like structure called the stipes (MS in figure 1), which carries a number of tactile hairs. The palpiger bears a large, singly innervated tactile hair (Ch3 in figure 2, Mx) (Schoonhoven & Dethier 1966).

The palp consists of three segments. The first carries one tactile hair (Ch4), two campaniform sensilla, and a 'free' nerve ending between the first and second segments. The second segment has a single campaniform sensillum and the third segment, four companiform sensilla and about nine basiconic sensilla (k1–k9). The innervation of each of these sensilla has not been
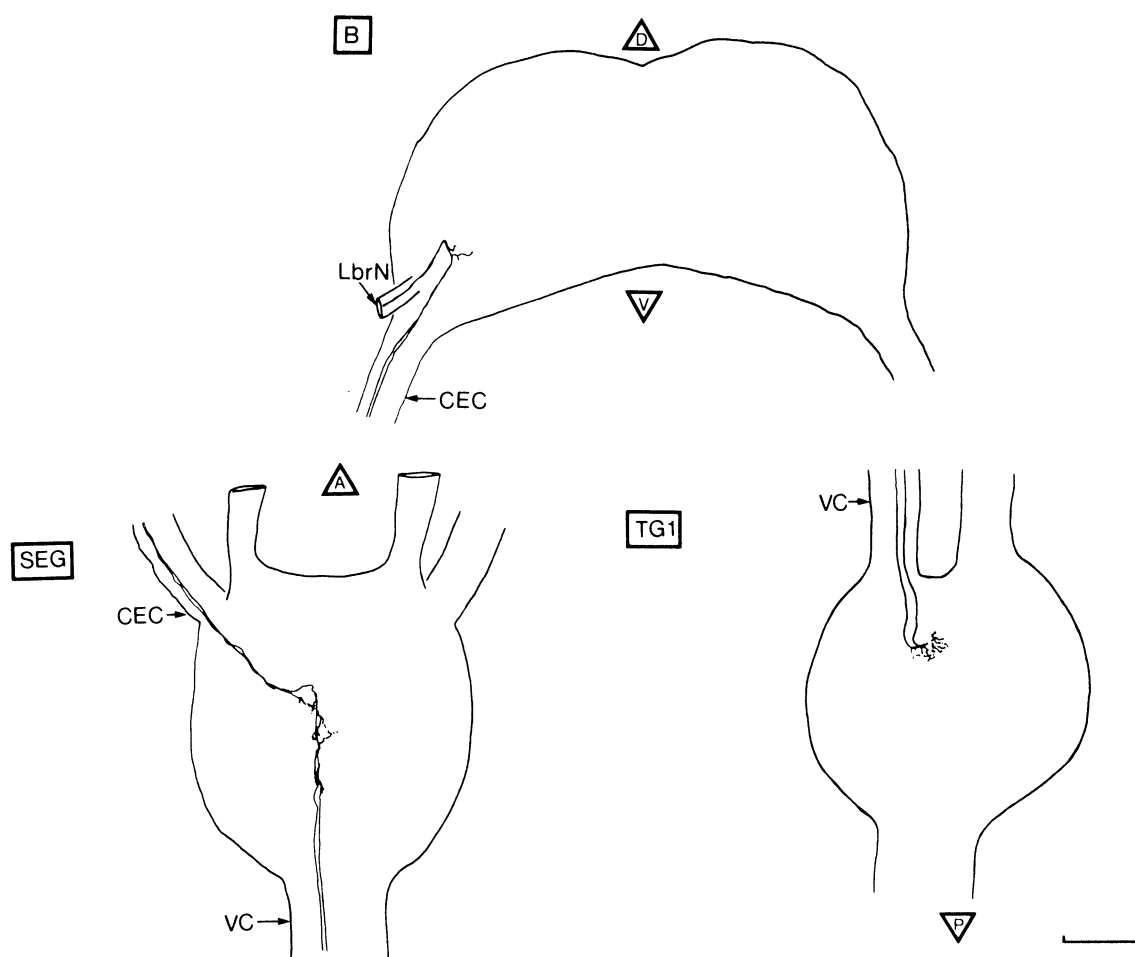
Figure 20. *Camera lucida* drawings of the brain (anterior view), SEG (ventral view), and TG1 (ventral view) showing fibres that stained after several tactile hairs on the clypeus had been cut and bathed in cobalt solution. Scale bar: 100 μm.

precisely determined in *Manduca*, but the third segment of the palp contains 19–24 cell bodies (Schoonhoven & Dethier 1966). At least some of the basiconic sensilla must be multiply innervated, since the five campaniform sensilla are singly innervated. Electrophysiological recordings showed two to four cells in each of these basiconic sensilla that were responsive to certain plant odours (Schoonhoven & Dethier 1966). Some cells may be mechanoreceptive or temperature-sensitive (Dethier 1941; Schoonhoven & Dethier 1966; Schoonhoven 1967; Dethier 1975). In larvae of other species of Lepidoptera, some of the basiconic sensilla are responsive to chemicals in solution (Schoonhoven & Dethier 1966), and in *Pieris* and *Manduca* five of these sensilla have a terminal pore characteristic of contact chemoreceptors (Ma 1972; Schoonhoven 1972).

The galea (Gl in figure 2, Mx) bears three tactile hairs (Ch5–Ch7), two campaniform sensilla, two *sensilla styloconica* (Z1, Z2), and two *sensilla basiconica* (GK1, GK2). The *sensilla styloconica* (Z1 and Z2) on the galea have been the most thoroughly studied electrophysiologically. In *Manduca* each is innervated by five neurons (Schoonhoven & Dethier 1966). Three or four of these cells appear to respond to gustatory stimuli such as nutrients, stimulants, or deterrents

(Schoonhoven & Dethier 1966), and three of the four cells in the lateral sensillum (Z2) respond to olfactory stimuli as well (Städler & Hanson 1975). One cell is apparently mechanosensory (Schoonhoven & Dethier 1966; Hanson 1970). The *sensilla basiconica* on the galea (GK2 and GK1) are innervated by one and three neurons respectively (Schoonhoven & Dethier 1966). Each of the tactile setae (Ch5–Ch7) is innervated by a single bipolar neuron and has been shown to be mechanosensory in *Manduca* (Hanson 1970). The campaniform sensilla are also singly innervated (Schoonhoven & Dethier 1966).

We selectively stained sensory neurons innervating maxillary sensilla by filling with cobalt after cutting a maxilla at the level of the palpiger, the galea, or the palp. In all cases, axons enter the SEG ventrally via the maxillary nerve (MN in figure 3). About halfway into the ganglion many of the fibres branch and form a rounded array of arborizations in ventrolateral neuropile (a in figure 21, SEG; figure 22). Several fibres turn through 90°, extend medially, and fork ipsilaterally near the midline of the ganglion, and some branches appear to cross over to the medial edge of the contralateral hemiganglion (b in figure 21, SEG, SEG(sag); figure 22). Some axons give rise to posteriorly directed processes, which put out many short branches along their length (c in figure 21, SEG, SEG(sag)). In some preparations, one long lateral process arises from one of these axons (d in figure 21, SEG). Histological sections demonstrated that the posteriorly directed processes travel in the core of neuropile that also receives antennal axons. In preparations in which the palp had been cut and filled with cobalt, one or a few stained, posteriorly directed fibres in the SEG projected to and terminated in ventral ipsilateral neuropile of TG1 (figure 21, TG1).

Other fibres that enter the SEG via the maxillary nerve do not run directly toward the midline of the ganglion but project dorsally, sending out a few short, medially directed branches on their way anterolaterally to the CEC (e in figure 21, SEG, SEG(sag)). Emerging from the CEC, these axons have arborizations in the tritocerebrum and deutocerebrum, extending into the region of the LAC (figure 21, B, B(sag); large arrow in figure 23, plate 3).

Attempts to fill neurons innervating only the *sensilla styloconica* on the galea revealed fewer stained fibres with projection patterns resembling those observed after the entire galea was cut and filled (figure 24). One of these axons has arborizations in the ventrolateral region of maxillary neuropile in the SEG and then extends medially, forking near the midline. In some preparations, another similar fibre stains but also has a posteriorly directed process. In addition, three or four fibres project to the brain. Because we cannot be certain that cobalt did not leak during filling of the styloconic sensilla, it is possible that fibres projecting to the brain may have come from other sensory neurons of the galea, such as those innervating the *sensilla basiconica* (GK1 and GK2).

When neurons innervating individual tactile hairs on the palpiger (Ch3), and galea (Ch5), or the palp (Ch4) were filled with cobalt, the stained axons were restricted to the SEG (figure 25a–c). These fibres enter the SEG via the maxillary nerve, have arborizations in the ventrolateral region, turn medially to ramify again, and usually (except the axon from Ch5 (figure 25c)) continue posteriorly, branching along their length and sending out one long lateral process. Tactile hairs located below the palpiger on the stipes of the maxilla are innervated by neurons whose axons ramify sparsely in the lateral, rounded region of SEG neuropile, turn medially and then posteriorly, and finally leave the SEG via the ventral connective and project to ventral neuropile in TG1 (figure 25d). We cannot rule out the possibility that some mechanosensory neurones might project to the brain because we did not
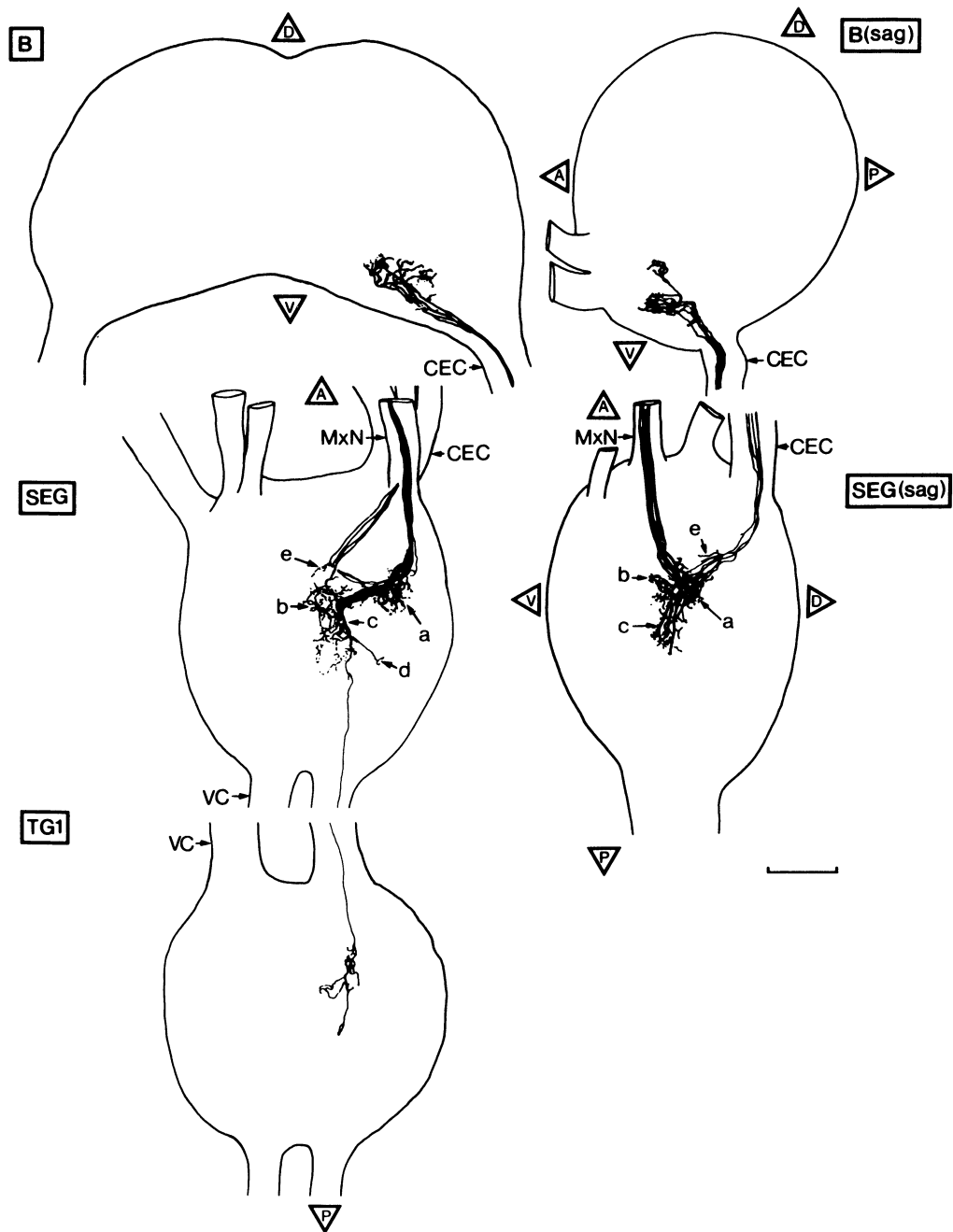
Figure 21. *Camera lucida* drawings showing fibres that stained after both palp and galea of the maxilla had been cut and exposed to cobalt solution. Anterior (B) and sagittal (B(sag)) views of the brain show fibres that enter from the CEC. Ventral (SEG) and sagittal (SEG(sag)) views of the SEG show fibres that enter via MxN. a, Rounded array of arborizations; b, fibres that fork at the midline and cross over to the contralateral side; c, arborizations of posteriorly directed processes; d, long lateral process that stains in some preparations. A ventral view of TG1 shows a fibre that stained in some preparations. Scale bar: 100 μm.
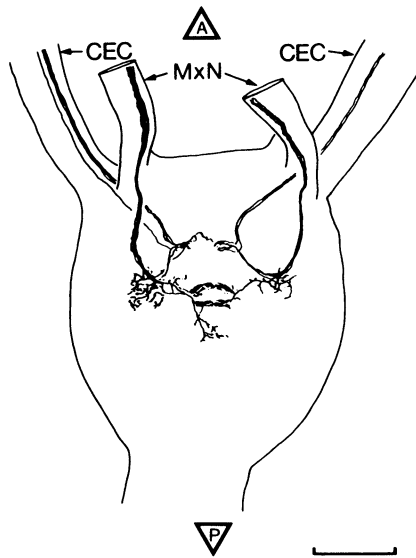
Figure 24. A *camera lucida* drawing of a ventral view of the SEG showing two different staining patterns achieved after a single *sensillum styloconicum* on each galea had been cut and bathed in cobalt solution. Scale bar: 100 μm.

fill Ch6 or Ch7 on the galea or single campaniform sensilla or 'free' nerve endings of the maxillae. Central projections of neurons innervating campaniform sensilla on the maxillary palp of the locust, however, are also restricted to the SEG and resemble those of neurons innervating Ch3 and Ch4 in *Manduca* (Bräunig *et al.* 1983).

In some animals we excised the palp in an earlier instar to cause degeneration of its sensory neurons. Cobalt staining of neurons innervating single *sensilla styloconica* on the galea of these animals still revealed a projection to the brain (figure 26, B), and, in most cases, fibres that had arborizations in the ventrolateral and ventromedial regions of the SEG (figure 26, SEG).

When both the palp and the galea had been excised in early instars, the stained maxillary nerve lacked fibres that project to the brain and exhibited only fibres that ramify within the SEG like those from the tactile hairs on the maxilla (Ch3, Ch4) or the maxillary stipes (figure 27). These ablation experiments confirmed that staining of fibres projecting to the brain is not caused by leakage of cobalt to sensilla on the palp, and thus supported the idea that fibres projecting to the brain arise from chemosensilla on the palp and galea.

The labial–hypopharyngeal pathway

The labial palps lie on either side of the base of the spinneret (Sp in figures 1 and 2, L-Hp) at the border of the labium and the hypopharynx (Hp in figures 1 and 2, L-Hp). Each palp appears to consist of a single segment bearing two hair-like sensilla (LP in figures 1 and 2, L-Hp). The medial side of each palp has a flattened cuticular extension (CE in figure 2, L-Hp). The hypopharynx is a tongue-like lobe, covered with spines or microtrichia (S in figure 2, L-Hp), that is fused with the labium and forms the base of the buccal cavity.

Projections from tactile hairs on the labium and from sensilla on the labial palp–spinneret complex enter the SEG via a nerve that is caudal to the maxillary nerve and somewhat thinner. This nerve in *Manduca quinquemaculata* was named 'z' by Peterson (1912) and described as an unidentified nerve that extends into a region near the salivary ducts. We traced this nerve into
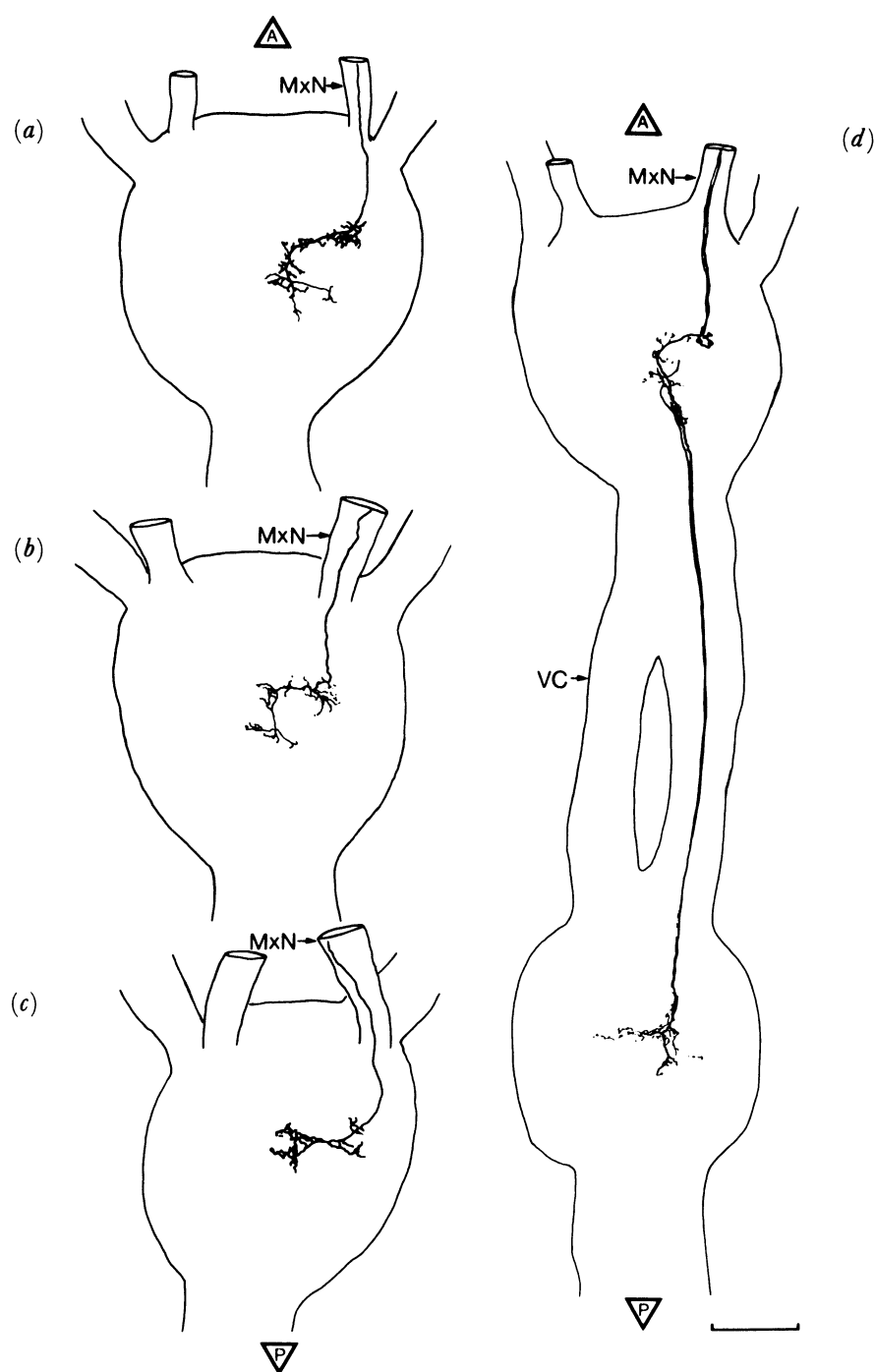
Figure 25. *Camera lucida* drawings of fibres arising from tactile hairs on the maxilla. (*a–c*) Ventral views of SEGs showing single fibres from tactile hairs on (*a*) the palpiger (Ch3), (*b*) the palpus (Ch4), and (*c*) the galea (Ch5). (*d*) A ventral view of the SEG and TG1 showing the staining pattern of fibres from tactile hairs on the maxillary stipes. Scale bar: 100 μm.
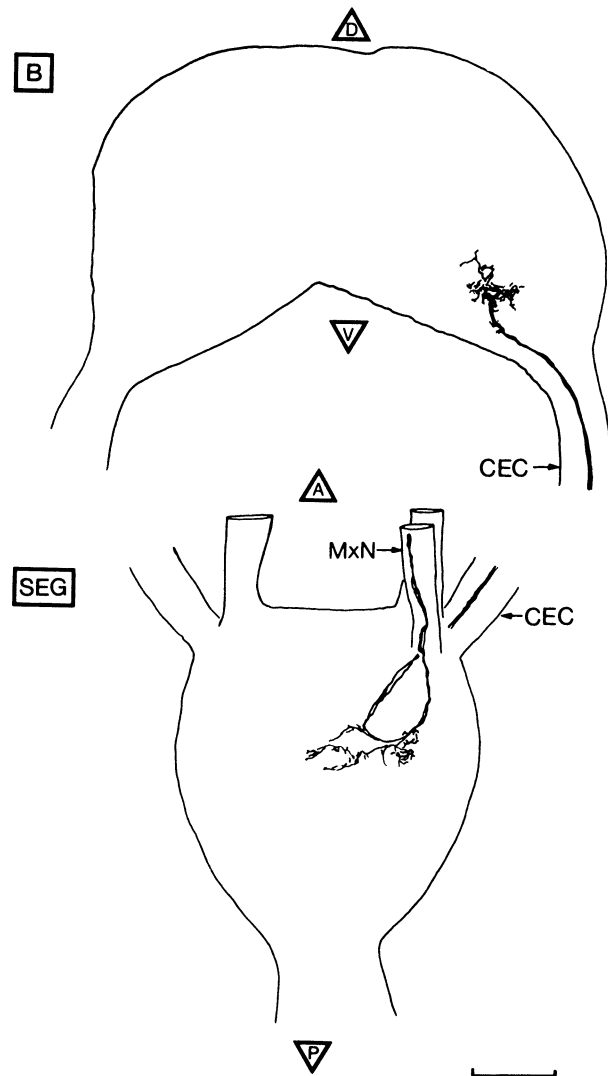
Figure 26. *Camera lucida* drawings of maxillary fibres that stained after second and third segments of the maxillary palp had been excised in an earlier instar. An anterior view of the brain and a ventral view of the SEG show the results of cutting a single *sensillum styloconicum* and exposing it to cobalt solution. Scale bar: 100 μm.

the labial palps and refer to it as the labial nerve (LN in figure 3) because fibres from the labium run in it.

Axons stained by filling a cut labial palp with cobalt solution enter the SEG ventrally and ramify sparsely in a region posterior to the maxillary region of neuropile (a in figure 28, SEG, SEG(sag); figure 29). The fibres turn, extend medially toward the midline of the ganglion, and give off anterior and medial processes, some of which cross the midline to the contralateral side of the ganglion (b in figure 28, SEG; figure 29). One or two fibres arise from anteriorly directed processes and travel anterodorsally, putting out short medially directed branches (c in figure 28, SEG, SEG(sag)) before running laterally to the CEC. These fibres pass through the CEC to the brain and terminate in a small, 'tufted' arborization near the posteroventral edge of the LAC (large arrow in figure 28, B; figure 30, plate 3)

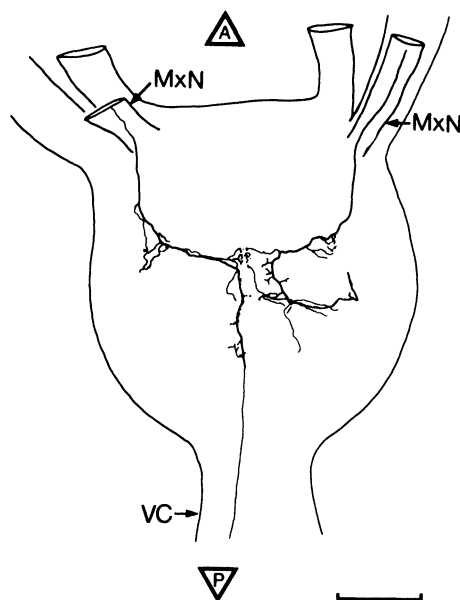Because we cut the tip of the labial palp to stain its innervation and were unable to fill cells

Figure 27. A *camera lucida* drawing of a ventral view of the SEG showing maxillary fibres that stained after both palps and galea had been excised in an earlier instar. The remaining maxillary palpiger was cut and bathed in cobalt solution. Scale bar: 100 µm.

associated with single sensilla, we cannot be sure which among the stained fibres were associated with the hair-like sensilla. Moreover, we may have stained neurons of campaniform or other mechanosensory sensilla elsewhere on the palp or labium.

We excised one or both labial palps to cause degeneration of the labial-palp sensory neurons. One or two instars later, we cut the spinnerets and associated cuticular structures and applied cobalt solution to them. The resulting pattern resembled that obtained by filling a normal labial palp with cobalt. The one fibre that normally projects to the brain was always absent (figure 31), however, suggesting that it arises within the palp. The fibres that did stain in the de-palped animals probably were from neurons innervating the base of the palp or the spinneret complex. The projection pattern of neurons innervating the tactile hairs on the palp may be similar to that of neurons innervating other mechanosensilla at the base of the palps or on the labium or hypopharynx; the lack of stained fibres from neurons innervating the tactile hairs on the palps therefore may go unnoticed in operated preparations. Hence we cannot conclude that the fibre projecting to the brain arises from a cell innervating one of the tactile hairs.

Neurons innervating tactile hairs on the post-mentum of the labium (L in figure 1) show a similar patten of projections when stained, but no fibres project to the brain. In addition to anteriorly directed branches, these axons have posteriorly directed processes that ramify sparsely in the SEG and then project through the ventral connective to form additional arborizations in ventral neuropile of TG1 (figure 32).

In addition to the fibres in the labial nerves, axons in a branch of the mandibular nerve (HN in figure 3) are stained in some preparations. These axons enter the SEG dorsally, dive to ventral neuropile, and send processes posteriorly, laterally, and medially (a in figure 33, SEG). The axons also bifurcate near the entrance of the mandibular nerve (MdN in figure 3) into the SEG (b in figure 33, SEG) and send a process through the CEC to the tritocerebrum to
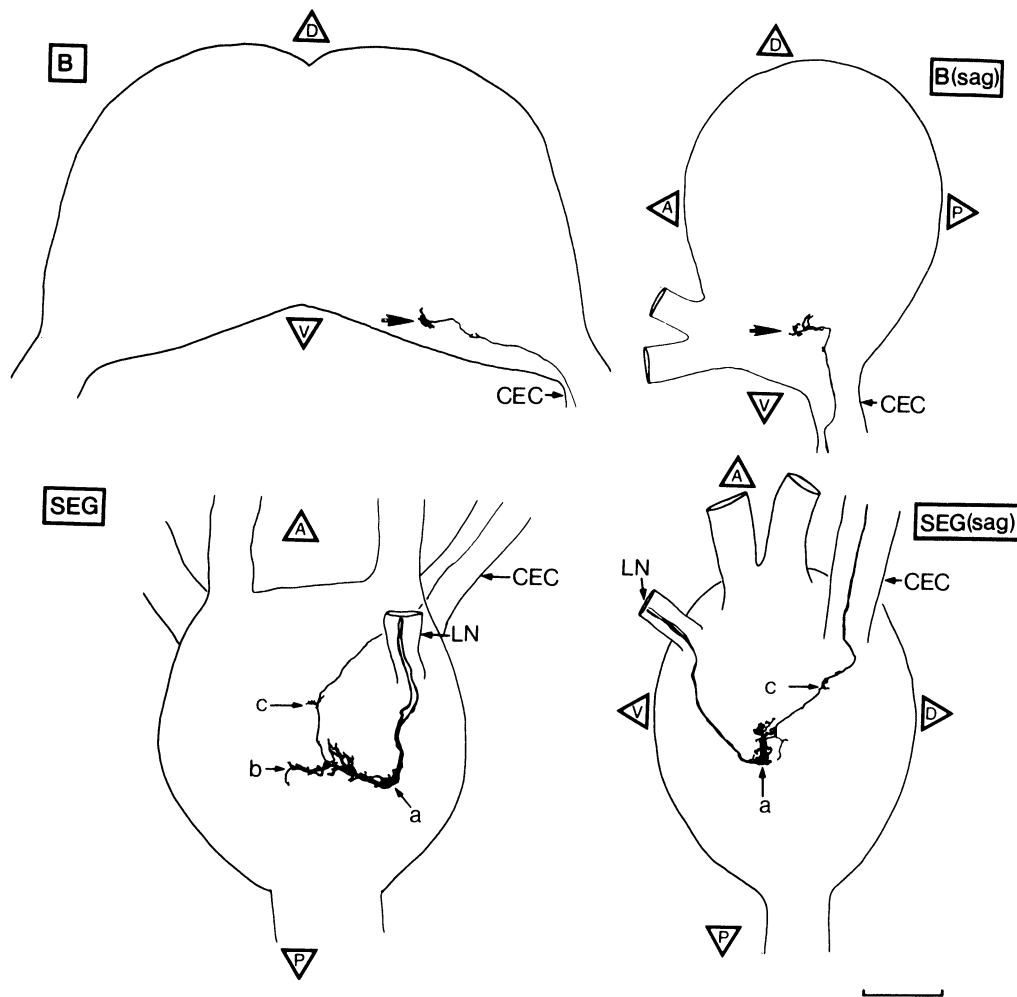
Figure 28. *Camera lucida* drawings of fibres stained after the labial palp had been cut and exposed to cobalt solution. Anterior (B) and sagittal (B(sag)) views of the brain show a fibre projecting to posterior side of the LAC (large arrow). Ventral (SEG) and sagittal (SEG(sag)) views of the SEG show fibres entering via LN. a, Arborizations of labial fibres as they turn to run medially; b, processes that cross the midline to the contralateral side of the SEG; c, short medially directed branches of the fibre travelling toward the CEC and to the brain. Scale bar: 100 μm.

form diffuse terminal arborizations very close to the entrance of the CEC into the brain (c in figure 33, B). We believe that these fibres arise from sensilla on the hypopharynx because they stain when parts of the hypopharynx are included among the structures exposed to cobalt solution. We traced this branch of the mandibular nerve to the region of the hypopharynx between the two labial palps and just proximal to the spinneret. Furthermore, we also observed this staining pattern in brains of animals whose labial palps had been excised in an earlier instar (figure 34, B, SEG). Peterson (1912) named this branch of the mandibular nerve the labial nerve. We find that labial axons pass through the unidentified nerve 'z' described by Peterson, and therefore suggest that nerve 'z' is the labial nerve. The branch of the mandibular nerve may correspond to the 'hypopharyngeal nerve' described in other insects (Snodgrass 1935).

Whether the projection in this hypopharyngeal nerve is chemosensory or mechanosensory cannot be determined from our study. It has been thought that the hypopharynx contains
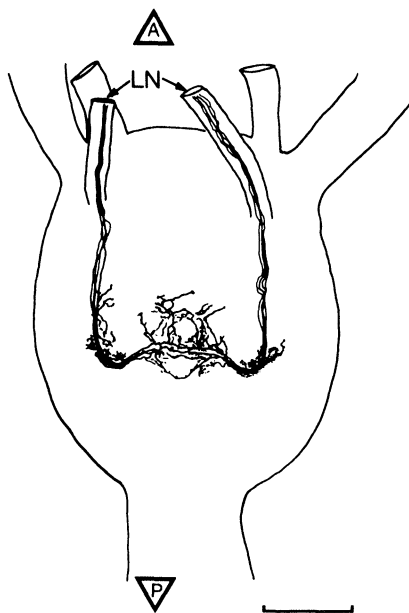
Figure 31. A *camera lucida* drawing of a ventral view of the SEG showing fibres that stained after the labial palps had been excised in an earlier instar. Staining was achieved by cutting across the spinneret complex and region where the labial palps would have been and exposing the entire area to cobalt solution. Scale bar: 100 μm.

gustatory sensilla, but none has been identified (Dethier 1975; De Boer *et al.* 1977). The only evident sensilla on the hypopharynx are small spines, and although they appear to be innervated (Dethier 1937), characterization of their receptor function awaits physiological and ultrastructural studies.

The mandibular pathway

Each mandible bears two tactile hairs on its outer surface (MdTH in figure 1). In addition, mandibular canal organs or scolopidial receptors with a proprioceptive function probably exist within each incisor region as in other lepidopterous larvae (Ma 1972; Albert 1980).

Neurons innervating tactile hairs on the mandible send axons through the mandibular nerve to the SEG. A fibre enters the SEG dorsally and passes ventrally to more central neuropile as it extends posteriorly, sending out stubby branches along the ipsilateral side of the midline of the SEG. A long lateral branch projects to more ventral neuropile at the level of the maxillary neuromere. The posteriorly directed process does not pass out of the SEG (figure 35). Histological sections demonstrated that this posteriorly directed process travels in the core of neuropile that receives antennal axons.

When we cut a tooth of the mandible and exposed it to cobalt solution, the axons that stained entered the SEG via the mandibular nerve and arborized in a region of neuropile anterodorsal to the maxillary projection (figure 36; figure 37, plate 3). A few fibres seemed to cross the midline to the medial region of the contralateral hemiganglion.

The stemmatal pathway

The larva has six simple eyes or stemmata (St in figure 1) on each side of the head lateral to the antennae. Visual cues probably influence selection of plants by some insects, and this
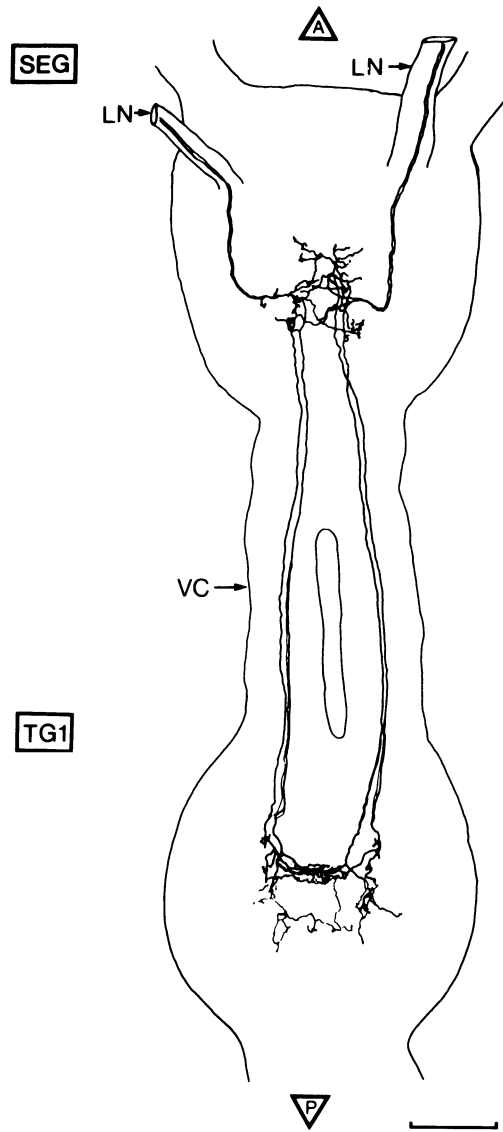
Figure 32. A *camera lucida* drawing of a ventral view of the SEG and TG1 showing fibres arising from tactile hairs on the post-mentum of the labium. Scale bar: 100 μm.

might be true as well for larval *Manduca*. We stained the stemmatal axons, however, as part of our effort to define sensory centres in the brain. We dipped the stemmatal nerve (SN in figure 3) in a pool of cobalt solution or pierced the stemmata and bathed them with cobalt solution. Stained axons entered the protocerebrum and terminated in blunt arborizations in the lateral neuropile of the ipsilateral protocerebrum (figure 38). This region of neuropile also receives input from branches of the fine fibres that emerge from the LAC and that stain after cobalt solution is applied to the cut end of the antenna (a in figure 38).
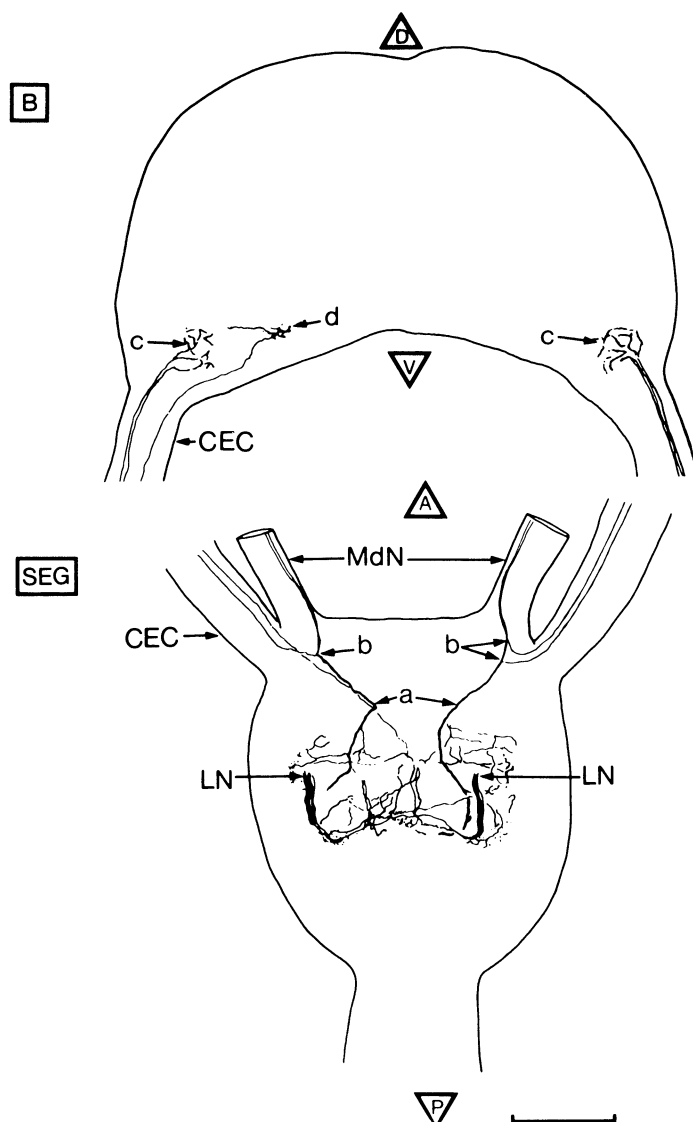
Figure 33. *Camera lucida* drawings of the brain and SEG showing additional fibres that stained after regions of the hypopharynx as well as the labial palps had been exposed to cobalt solution. Fibres enter the SEG via MdN from a branch (HN) that is beyond the limits of the drawing. a, Coarse fibres that send out many medial and lateral branches; b, point at which fibres bifurcate to send processes to the brain; c, termination of 'hypopharyngeal' fibres lateral to the process arising from the labial palp, d. Labial nerves (LN) were cut at points of attachment to the SEG to allow visualization of branching processes. Scale bar: 100 μm.

DISCUSSION

The neuropile of the primary olfactory centre in many adult insects is glomerular (Bullock & Horridge 1965). Each glomerulus is a region of synaptic interaction among primary-afferent olfactory axons and olfactory interneurons (Boeckh *et al.* 1970; Schürmann & Wechsler 1970; Masson 1972; Tolbert & Hildebrand 1981). The complexity of olfactory neuropile in holometabolous larvae has been correlated with the complexity of the larval antenna (reviewed by Edwards 1969). In larvae of *Danaus* and *Tenebrio*, the olfactory neuropile is glomerular. In
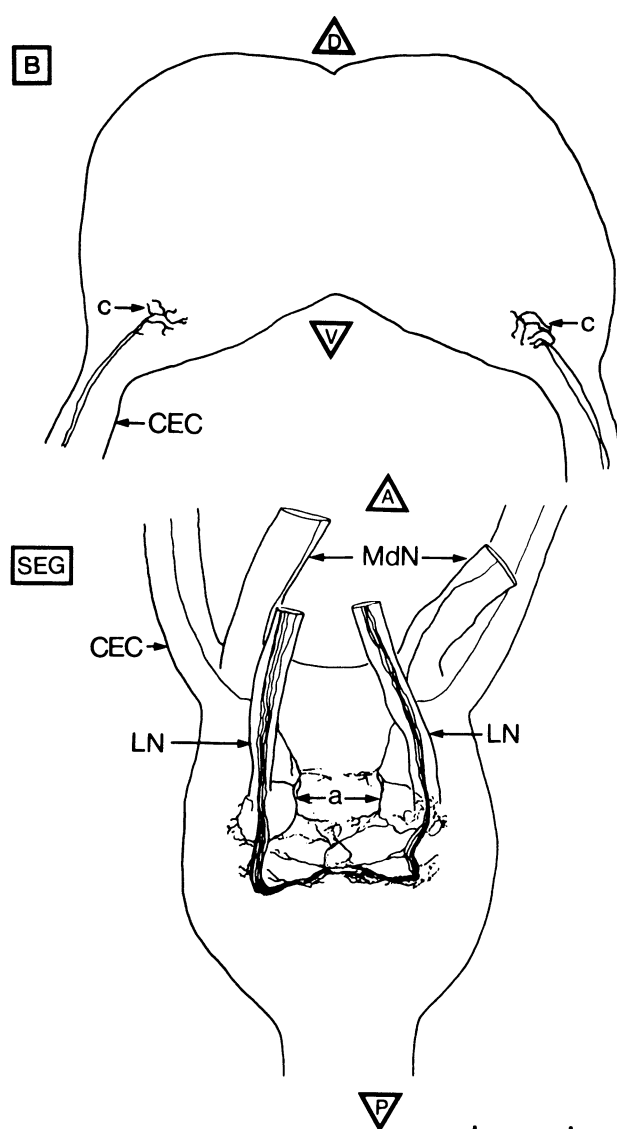
Figure 34. *Camera lucida* drawings of the brain and the SEG of a preparation similar to that of figure 33. In this preparation, the labial palps had been excised in an earlier instar. Coarse fibres, a, that enter via a branch of MdN are still present, as are processes projecting to the brain, c. Scale bar: 100 μm.

larvae with rudimentary antennae (such as bees, mosquitoes, and flies), the olfactory neuropile is not glomerular and may be suppressed or absent, particularly at early larval stages. In larval *Manduca*, we find that the antennal centre is glomerular or 'nodular' and that the axons of neurons that apparently innervate chemosensilla on the antenna terminate in this neuropile with 'tufted' processes.

The maxillary palps and galeae are the only other structures on the larval head known to contain sensilla responsive to odours. These maxillary sensilla are innervated by neurons whose axons project to regions of the deutocerebrum in and near the LAC. Perhaps neurons that respond to olfactory stimuli and innervate the *sensilla styloconica* (Städler & Hanson 1975) or the *sensilla basiconica* (Schoonhoven & Dethier 1966) on the maxillae project to the deutocere-
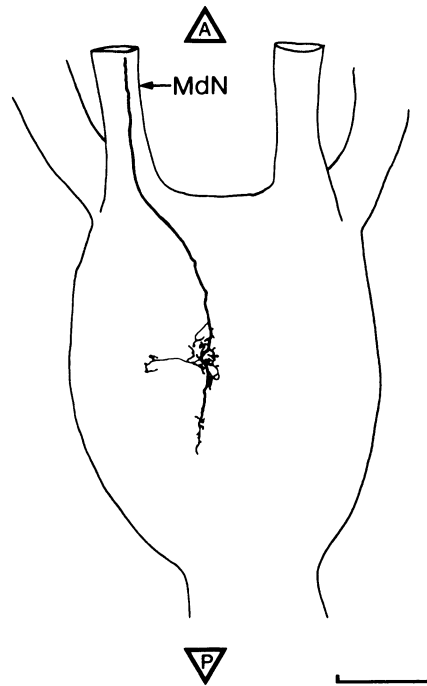
Figure 35. A *camera lucida* drawing of a ventral view of the SEG showing a single fibre from a tactile hair on the mandible entering the SEG via MdN. Scale bar: 100 μm.
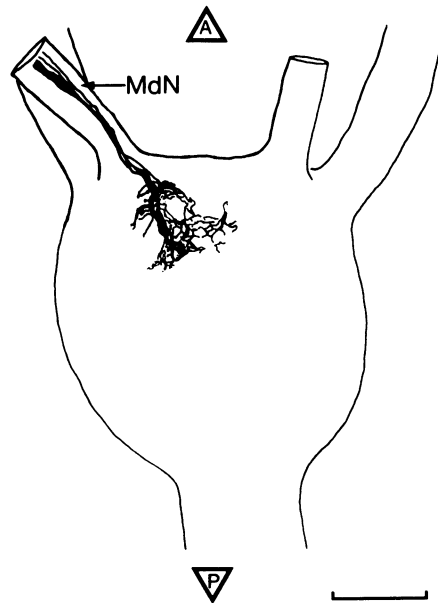
Figure 36. A *camera lucida* drawing of a ventral view of the SEG showing fibres that stained after mandibular teeth had been cut and exposed to cobalt solution. Scale bar: 100 μm.

brum and form synapses with interneurons that also receive inputs from the antennal olfactory sensilla.

While the olfactory receptors on the antenna are thought to guide the larva in short-range orientation toward plants (Dethier 1941; Ishikawa *et al.* 1969; Ma 1976), the olfactory receptors on the maxillary palp seem to facilitate feeding responses rather than to influence
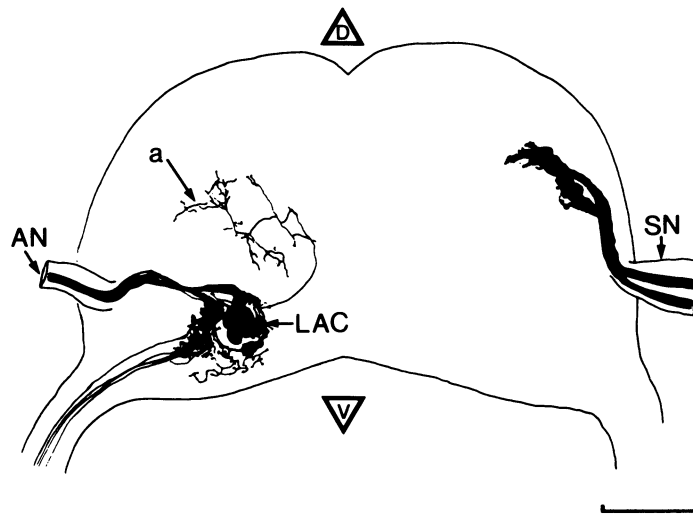
Figure 38. A *camera lucida* drawing of an anterior view of the brain showing fibres (right) that stained after the stemmata had been pierced and exposed to cobalt solution. These fibres enter the brain via SN. On the left side are fibres that stained after the cut antenna had been exposed to cobalt solution. Some of the branches of fine fibres, a, that emerge from the LAC are in the same region of the protocerebrum that receives input from stemmatal axons. Scale bar: 10 µm.

orientation behaviour, at least in the silkworm *Bombyx mori* (Ishikawa *et al.* 1969). Both of these behaviours might be influenced by sensory inputs to the LAC. Alternatively, some of the receptors on the maxillae may influence orientation toward a food source via the LAC, while others projecting to 'gustatory' regions of the tritocerebrum or SEG could facilitate feeding responses. Whether inputs from gustatory and olfactory receptors overlap or are segregated in the CNS is not clear from our observations and may require electrophysiological studies of the central neuropile.

Although removal of labial palps does not alter feeding behaviour in *Manduca* (Waldbauer & Fraenkel 1961; Waldbauer 1963), the projection of a single fibre to the brain in or near the LAC suggests a possible chemosensory input from the palps. The structure and innervation of sensilla on the labial palps of larval *Manduca sexta* have not been described. The two hair-like sensilla probably have a tactile function, but small chemosensilla, possibly associated with the flattened cuticle attached to the medial side of the palp, might also be present. The labial palps of the spruce budworm contain two peg sensilla that are presumably mechanosensory (Albert 1980). Other insects have chemosensory-like sensilla on their labial palps (Peregrine 1972; Ramaswamy & Gupta 1981). In the black cutworm, scanning electron microscopy shows two protuberances on the labial palps that the authors suggest may be either chemosensory or mechanosensory (Reese & Carlson 1974). The labial palps of adult *Drosophila* have external taste bristles and taste pegs. Axons from neurons innervating these sensilla terminate in the SEG, however, and do not overlap with projections from antennal axons (Stocker & Schorderet 1981). In contrast, the third segment of the labial palp of adult *Manduca* contains a pit or depression housing numerous peg-like sensilla (resembling olfactory or gustatory sensilla) innervated by neurons whose axons project to a single glomerulus in the antennal lobe (Harrow *et al.* 1983; Kent *et al.* 1986).

Gustatory receptors have been located on the maxillae and epipharynx of lepidopterous
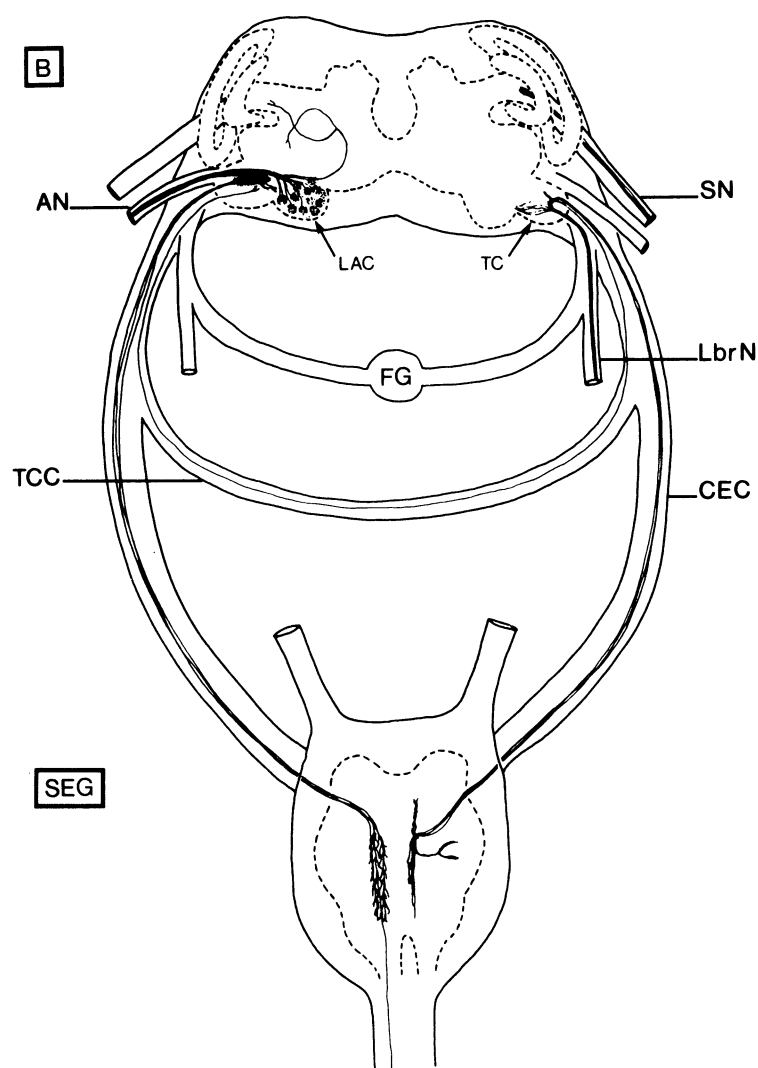
Figure 39. Summary diagram of the brain (anterior view) and SEG (ventral view) illustrating projections of sensory axons from the antenna, stemmata, and labrum. Many axons from the antenna project through AN to the LAC in the deutocerebrum. At least one axon passes beyond the LAC and has arborizations in various parts of the protocerebrum, including regions that receive projections of stemmatal axons (via SN). Some antennal axons pass lateral to the LAC and project to the SEG through the CEC. Axons from the labrum pass into the tritocerebral region (TC) of the brain via LbrN. Some axons pass through the CEC to the SEG. At least one axon passes through the TCC to the contralateral tritocerebrum.

larvae. Unlike olfactory neuropile, gustatory neuropile has not been well defined in insects. Based on response spectra of maxillary and epipharyngeal receptors and on ablation and behavioural studies in lepidopterous larvae, Ma (1972, 1976) suggested that maxillary input to the SEG facilitates feeding by arresting locomotion and evoking biting. Feeding incitants, he proposed, stimulate the maxillary receptors that evoke such responses. Our results confirm that maxillary sensilla are innervated by neurons that have projections in the SEG. Maxillary projections to the brain (deutocerebrum and tritocerebrum), however, may be from gustatory as well as from olfactory receptors. Projections from the maxillae to the tritocerebrum have been reported in the locust (Aubele & Klemm 1977; Ernst *et al.* 1977) and in the cockroach (Ernst *et al.* 1977).
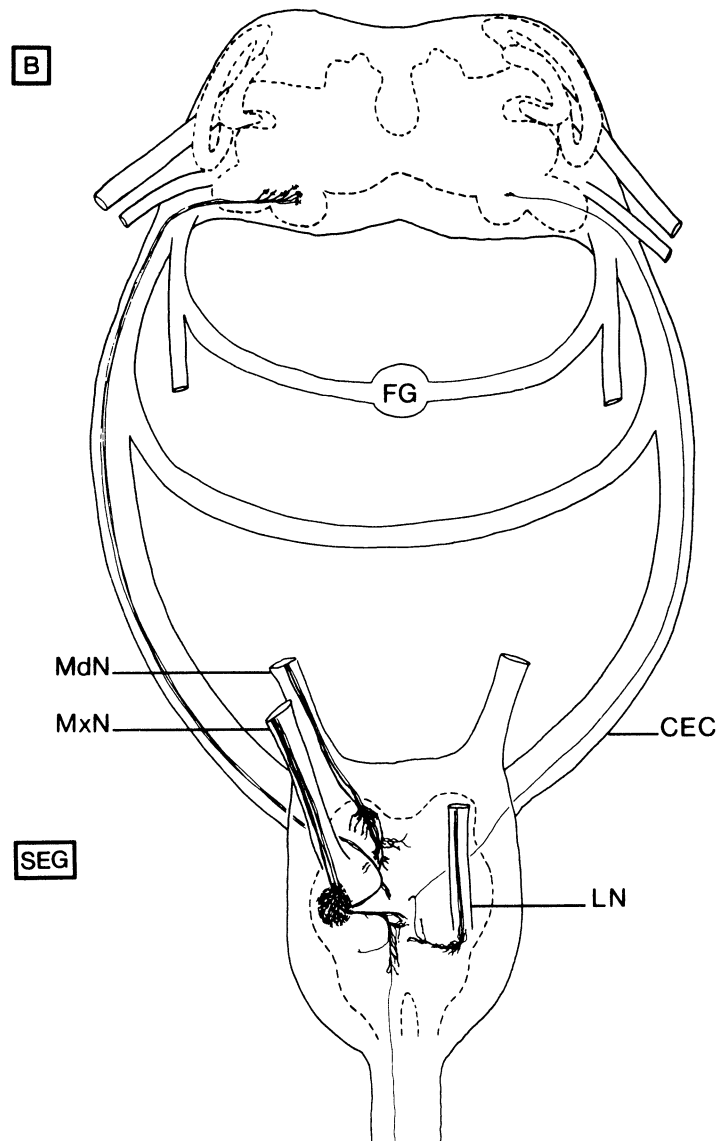
Figure 40. Summary diagram of the brain (anterior view) and the SEG (ventral view) illustrating central projections of sensory axons from the teeth of the mandible, the maxilla, and the labial palps. The mandibular axons occupy the mandibular neuromere of the SEG. Maxillary axons project into the maxillary neuromere of the SEG, and some fibres pass through the CEC to regions of the tritocerebrum and deutocerebrum. Labial axons project into the labial neuromere of the SEG, and at least one fibre passes through the CEC to the posterior side of the deutocerebrum.

In *Pieris*, feeding stimulants such as sugars stimulate epipharyngeal receptors in addition to maxillary receptors. Ma (1972, 1976) suggested that input from these receptors promotes ingestional movements, including swallowing. De Boer *et al.* (1977) determined that the epipharyngeal sensilla in *Manduca* respond to feeding deterrents but not to feeding stimulants. Those authors suggested that the epipharyngeal sensilla act as the final checkpoint in discriminating between host and non-host plants.

Ma (1972) claimed that afferent impulses from the epipharyngeal sensilla in *Pieris* are propagated to the anterior stomatogastric nervous system (frontal ganglion, hypocerebral

ganglion and gastric nerves), which controls movement of the gut and presumably swallowing. Our results suggest that axons from the epipharyngeal sensilla in *Manduca* larvae project to the tritocerebrum and possibly to the SEG as well. We have obtained no evidence for a direct input from the epipharyngeal sensilla to any part of the stomatogastric nervous system. Epipharyngeal receptors, responding to feeding deterrents, might inhibit swallowing via their direct projections to the tritocerebrum and perhaps polysynaptic pathways to the stomatogastric nervous system. Alternatively, motorneurons controlling swallowing may have dendritic arborizations in the tritocerebrum. In the cricket, neurons with somata in the frontal ganglion have arborizations in the tritocerebrum and send axons to buccal musculature. Furthermore, motorneurons with somata and dendritic arborizations in the tritocerebrum innervate labral compressor muscles via axons travelling in the frontal connectives, frontal ganglion and frontal nerves (Kirby *et al.* 1984).

Our cobalt staining of the neurons in the region of epipharyngeal sensilla also demonstrated processes that project to a region of SEG neuropile that is near or within the maxillary neuromere and within the central core of neuropile receiving mechanosensory projections from many areas of the mouth and head. Because more than three fibres often stained when we applied cobalt to the region of epipharyngeal sensilla, we cannot determine from the present results whether the processes reaching the SEG do indeed come from the epipharyngeal sensilla. If the chemosensory axons do project to the SEG, then the central regions involved in integrating input from the epipharyngeal sensilla must include the SEG as well as the tritocerebrum. In the cricket there is some evidence that neurons with somata in the SEG send axons through the CEC and brain to the frontal connectives, frontal ganglion, and frontal nerve apparently to innervate labral musculature (Kirby *et al.* 1984). Input to the SEG could therefore also directly influence motorneurons involved in swallowing.

Although we have observed no afferent projection from the region of epipharyngeal sensilla into the deutocerebrum, it is difficult to determine the boundaries of neuromeres near their points of fusion. Aubele & Klemm (1977) reported inputs from the labrum of the locust to the tritocerebrum, deutocerebrum, and protocerebrum. In adult *Drosophila*, axons from neurons innervating internal mouthparts (the labral sense organ on the epipahyrnx, the dorsal and ventral cibarial sense organs on the sucking apparatus) terminate in the tritocerebrum (Stocker & Schorderet 1981).

Projections from sensilla on the hypopharynx may also be involved in stimulation or inhibition of swallowing via the tritocerebrum. Whether these sensilla are chemosensory or mechanosensory is unknown. Proprioceptive feedback to tritocerebral or suboesophageal motorneurons controlling feeding is probably part of the input from the hypopharynx.

Mechanosensory receptors are located all over the head and body of insects. Central projections of axons from a variety of mechanosensory receptors on the head, antennae, and mouthparts of locusts have been described. Many of the central profiles in *Manduca* resemble mechanosensory profiles in the locust (Tyrer *et al.* 1979; Bräunig *et al.* 1983).

In all cases, neurons innervating single tactile hairs on the antennae, labrum, or head cuticle send axons through the brain to the SEG, where they have arborizations in a core of neuropile near the medial edge of the ipsilateral hemiganglion, traversing the maxillary and labial neuromeres as they run in a dorsal-to-ventral direction. In some cases, fibres project further to the ipsilateral anteroventral quadrant of TG1. Cobalt staining of neurons innervating tactile hairs on the mandibular, maxillary, and labial segments of the head also demonstrates

arborizations in the same core of SEG neuropile, in some cases with projections to the same region of TG1. Such overlapping projections suggest a common function, which may be concerned with orientation of the head, biting, and locomotion. These behaviours could facilitate feeding as well as playing a role in defence.

The region of antennal mechanosensory neuropile has been defined in adult *Manduca* and several other insects. Mechanosensory axons terminate and motor neurons have dendritic arborizations within the region referred to as the 'posterior antennal centre', 'mechanosensory and motor centre' or 'dorsal lobe' of the deutocerebrum, which is distinct from the glomerular neuropile of the antennal lobe (Sánchez 1937, 1941; Masson 1972; Suzuki 1975; Camazine & Hildebrand 1979; Hildebrand *et al.* 1980; Stocker *et al.* 1976; Stocker & Lawrence 1981; Strausfeld & Bacon 1982). In the larva, the coarser mechanosensory fibres, medial arborizations, and tufted process abutting the posterior side of the LAC might delineate a region of the deutocerebrum that corresponds to the adult mechanosensory and motor centre.

Based on their position on the incisive areas and the molar of the mandible, the mandibular canal organs were thought to have a chemosensory function (Zacharuk 1962; Le Berre & Louveaux 1969). Because ultrastructural studies demonstrated that the canals are not open at their distal ends (Corbière-Tichané 1971; Ma 1972), a gustatory function seems unlikely. Instead, these sensory organs resemble scolopidial receptors, which serve a proprioceptive role. Our cobalt staining of the innervation of such receptors failed to demonstrate processes extending to any of the putative chemosensory regions of neuropile and therefore lends support to the idea of a mechanosensory function.

We conclude that primary chemosensory neuropile occurs in the deutocerebrum, the tritocerebrum, and the maxillary neuromere of the SEG. These chemosensory regions may be further subdivided into olfactory neuropile (deutocerebrum) and gustatory neuropil (tritocerebrum and SEG), although we cannot confirm this hypothesis solely on the basis of our present anatomical evidence. Mechanosensory inputs from all over the head and mouthparts, particularly from tactile hairs, appear to overlap in a core of neuropile in the SEG, probably influencing both feeding and defensive behaviours.

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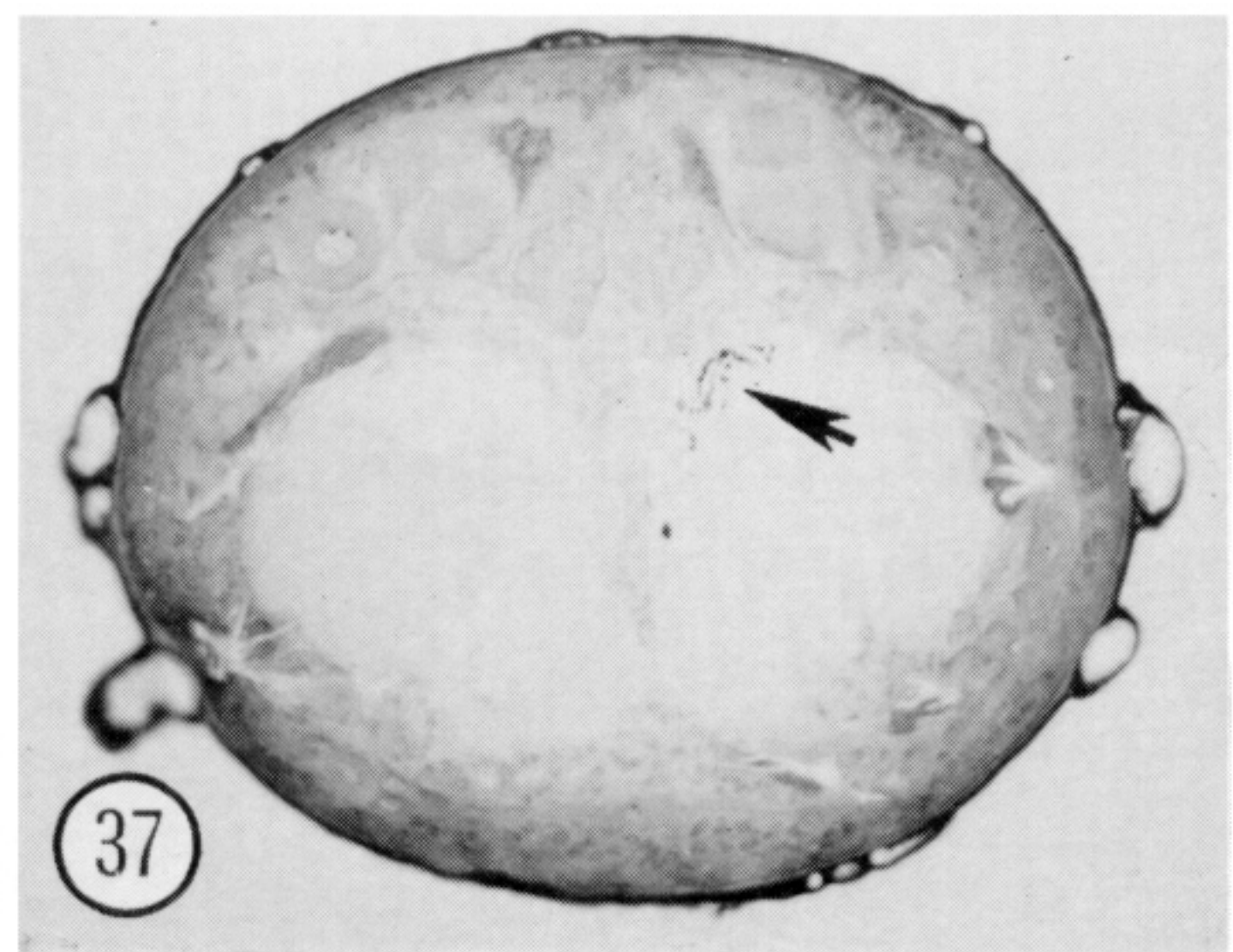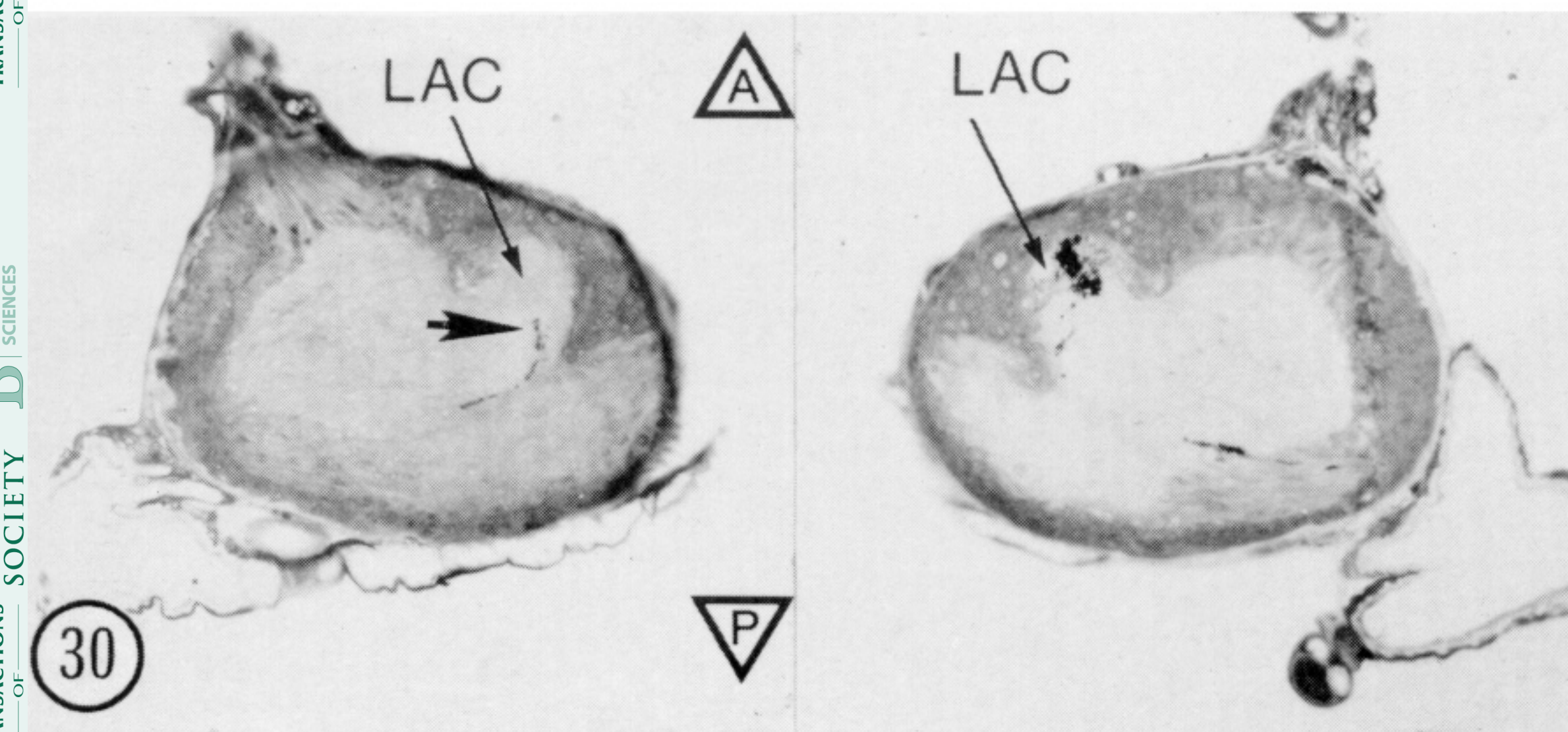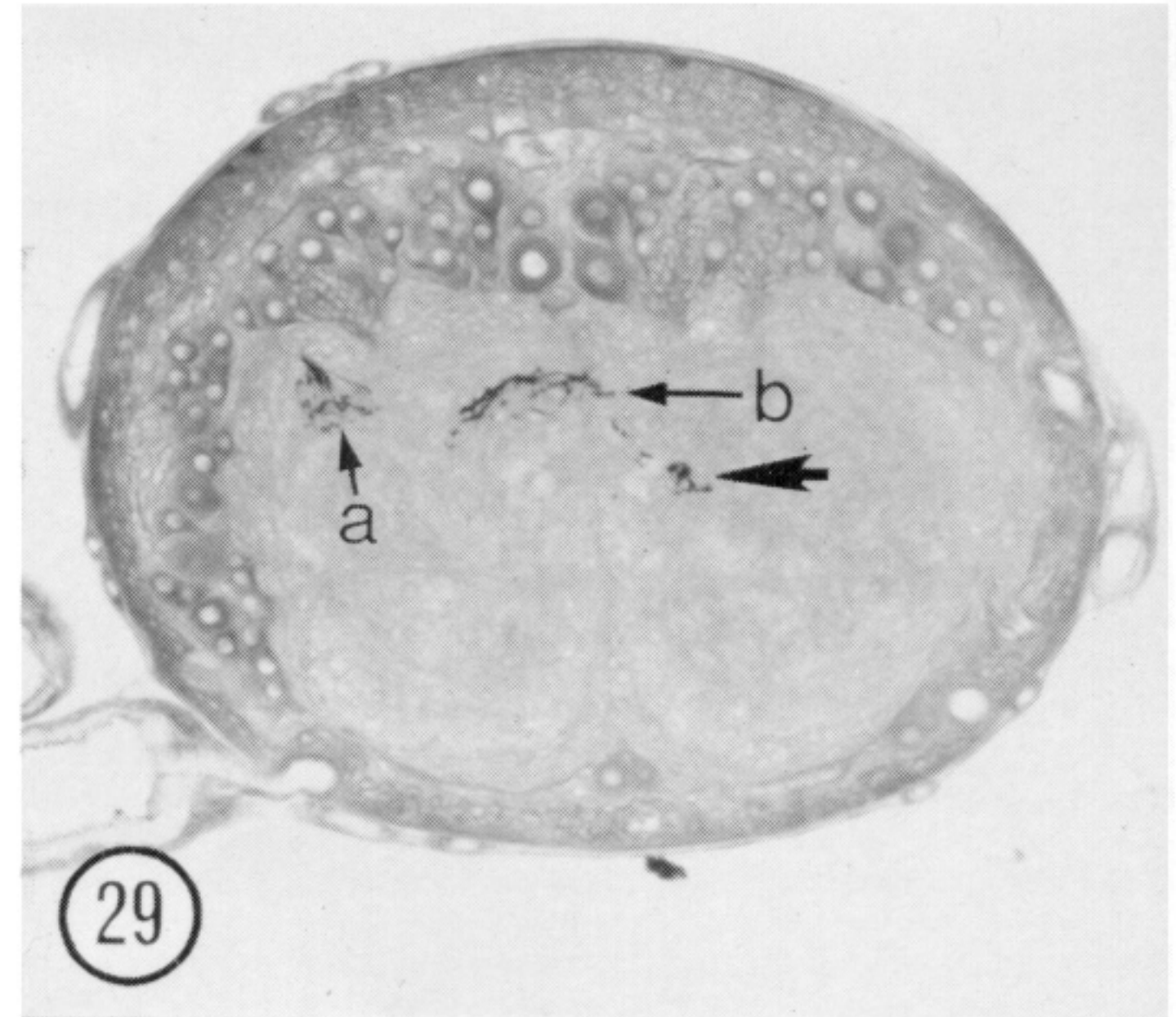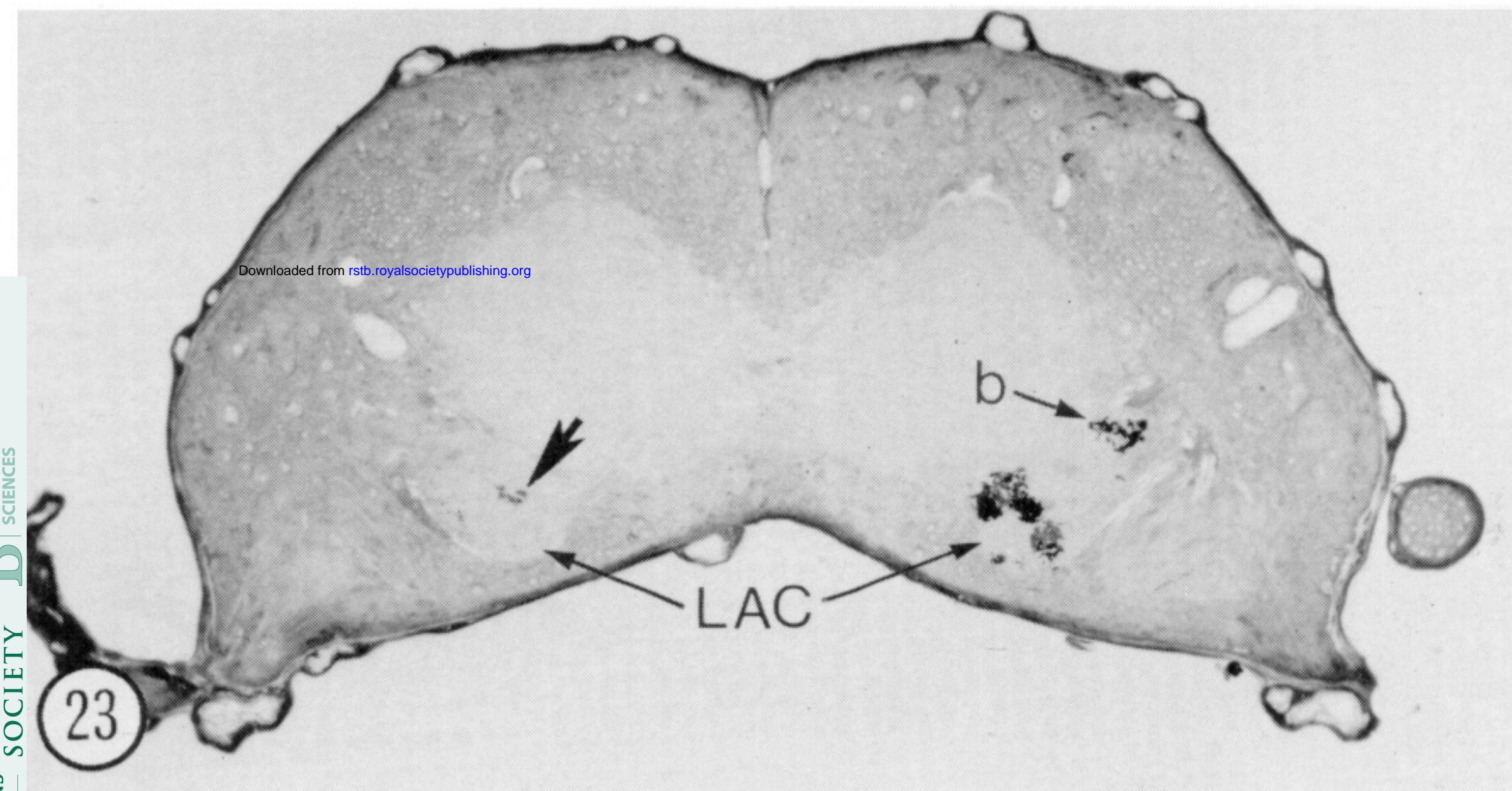
EXPLANATIONS OF ABBREVIATIONS

A	anterior	MdN	
B	brain	MdTH	tactile hair on mandible
C	*sensilla campaniformia*	Mx	maxilla
Ch1–Ch7	tactile hair on antenna or maxilla	MxN	maxillary nerve
CE	cuticular extension of labial palp	Mp	maxillary palpiger
CEC	circumoesophageal connective	O	oesophagus
D	dorsal	OA	anlage for adult optic lobe
ES	epipharyngeal sensilla	ON	optic neuropil
FC	frontal connective	P	posterior
FG	frontal ganglion	PC	protocerebrum
Gl	galea	PmI, PmII, PmIII	first, second, third segments of maxillary palp
GK1–GK2	*sensilla basiconica* of the galea		
HN	hypopharyngeal nerve	S	spines
Hp	hypopharynx	sag	sagittal
I, II, III	first, second, third segment of the antenna	SEG	suboesophageal ganglion
		SN	stemmatal nerve
K	large *sensilla basiconica*	Sp	spinneret
k	small *sensilla basiconica*	St	stemmata
L–Hp	labial–hypopharyngeal complex	TC	tritocerebrum
LAC	larval antennal centre	TCC	tritocerebral commissure
Lbr	labrum	TG1	first thoracic ganglion
LbrN	labral nerve	TH	tactile hair
LD	lateral domes	TS	tactile setae on epipharynx
LN	labial nerve	V	ventral
LP	labial palp	Z	*sensilla styloconica*
MB	mushroom bodies	1–8	*sensilla campaniformia* on antenna or maxilla
	mandibular nerve		

FIGURE 1. Scanning electron micrograph of the ventral surface of the head of fifth-instar larval *Manduca sexta*. A, antenna; Hp, hypopharynx; Lbr, labrum; L, post-mentum of labium; LP, labial palp; Mx, maxilla; MS, maxillary stipes; Md, mandible; MdTH, tactile hair on mandible; Sp, spinneret; St, stemmata; TH, tactile hair on head. Scale bar: 500 μm.
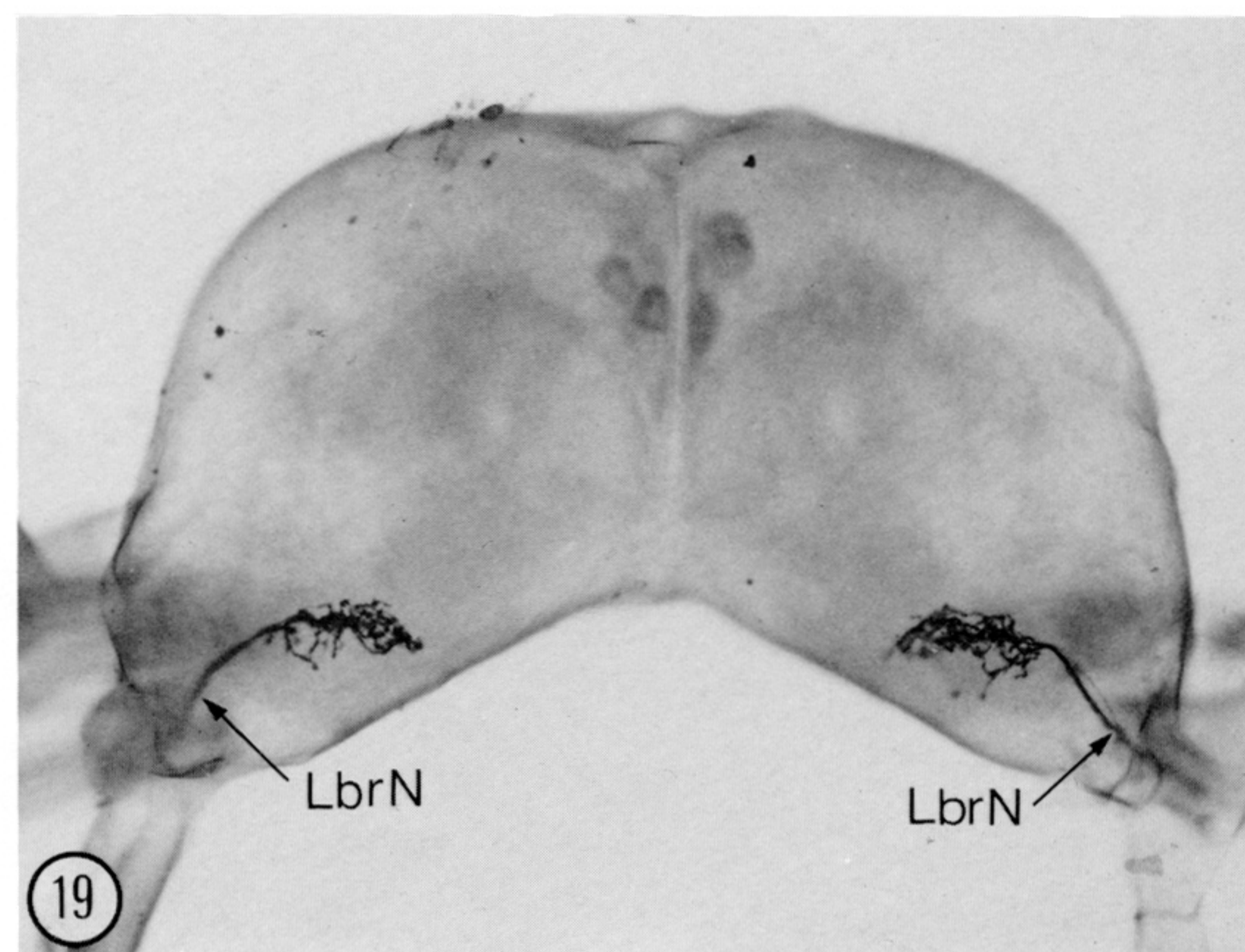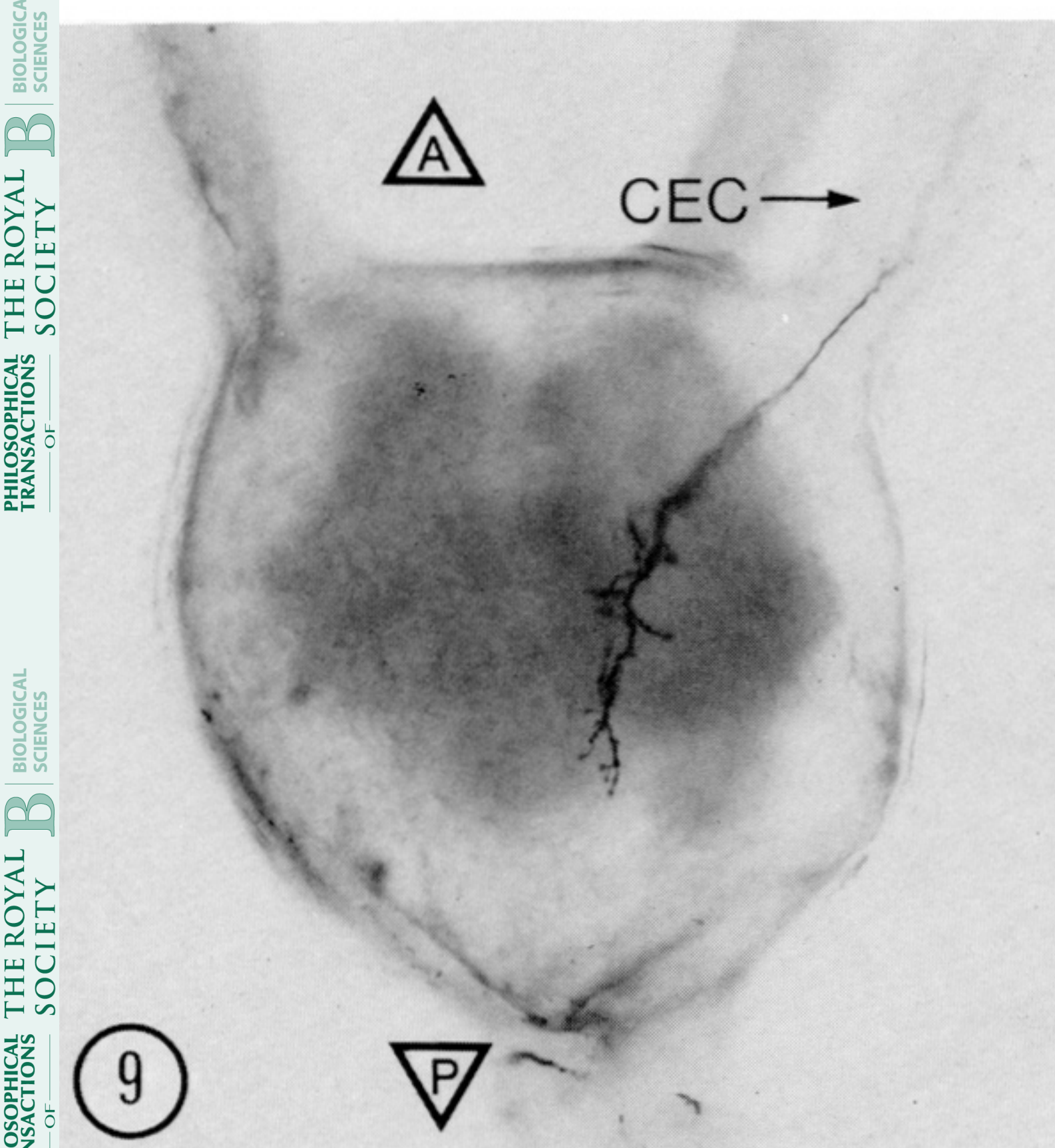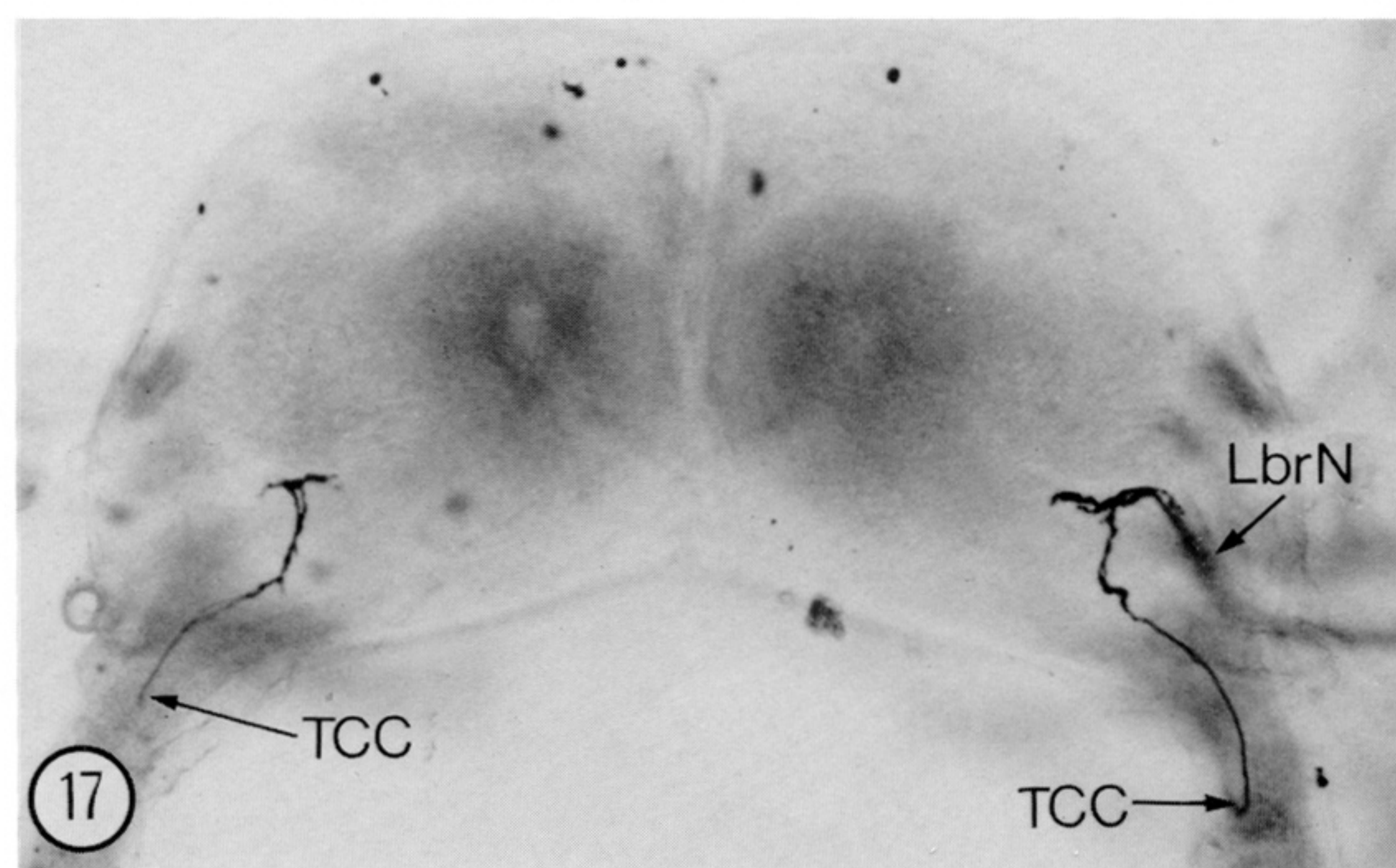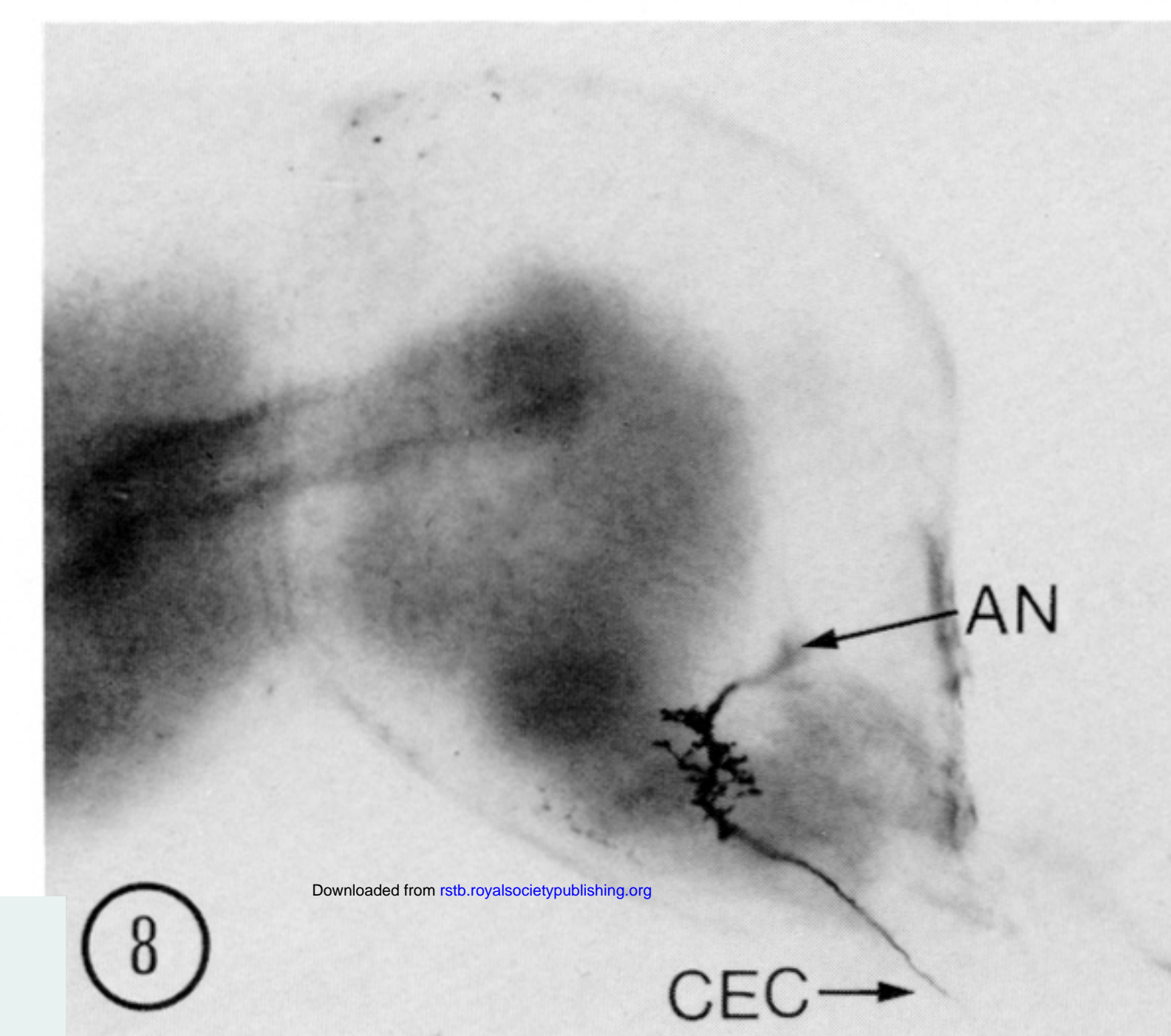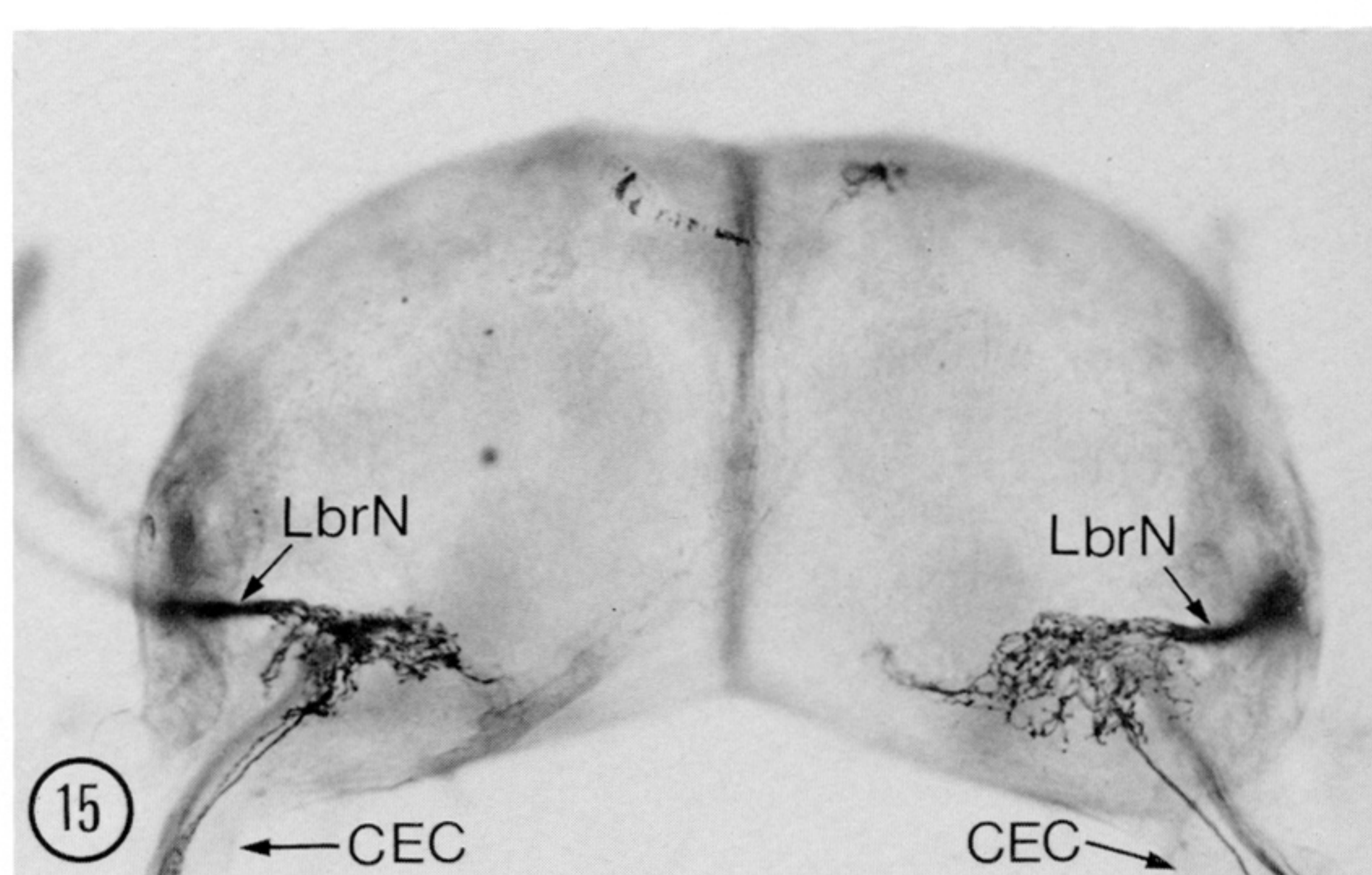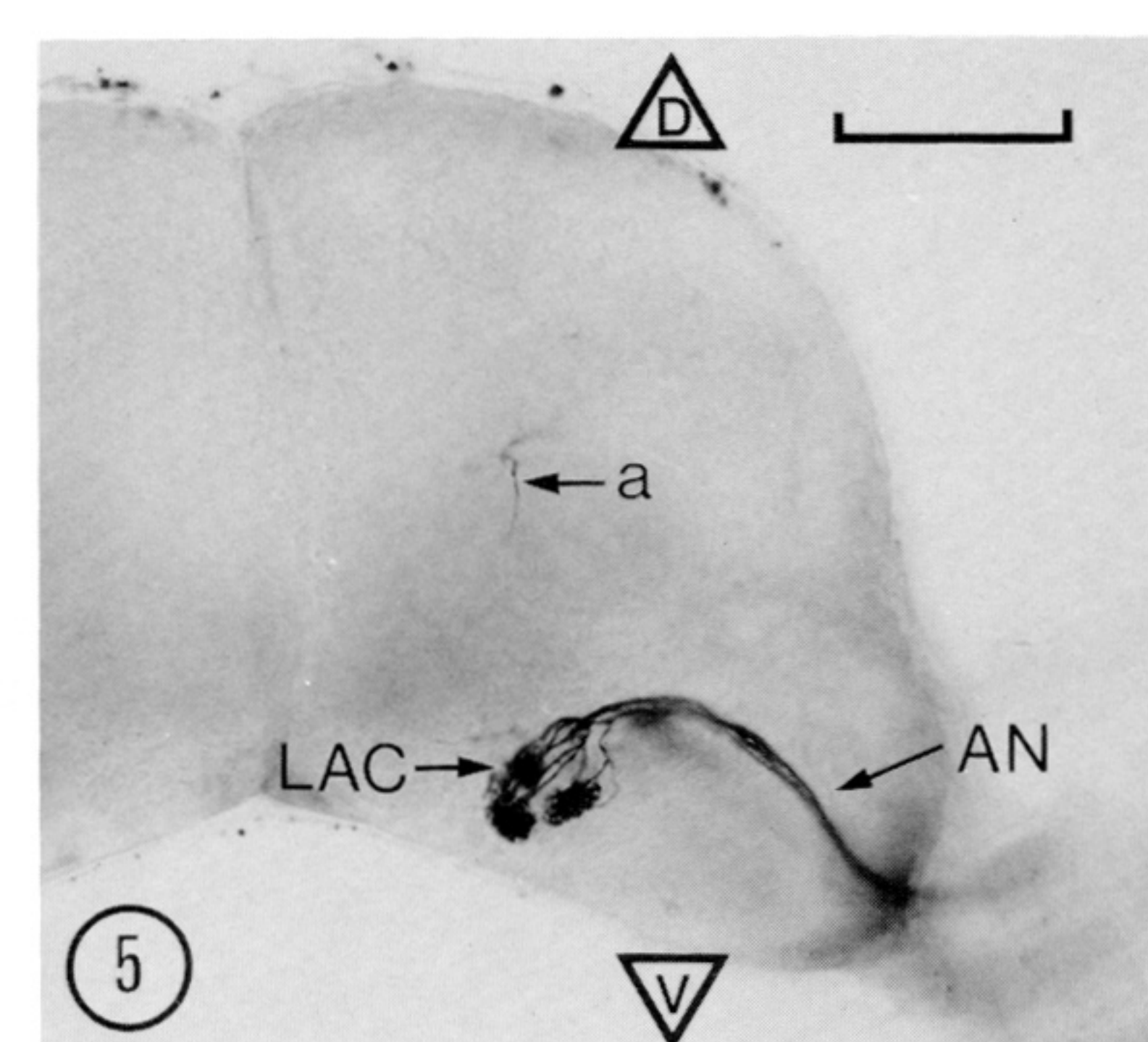
FIGURES 5, 8, 9, 15, 17 AND 19. For description see opposite.

FIGURES 6, 22, 23, 29, 30 AND 37. For description see opposite.