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Neuroarchitecture of the lower division of the central body in the brain of the locust (*Schistocerca gregaria*)

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Abstract. We have investigated the anatomical organization of the lower division of the central body in the brain of the locust *Schistocerca gregaria*. Bodian preparations, Golgi impregnations, and intracellular filling with Lucifer yellow have revealed that the lower division of the central body is organized into six horizontal layers and sixteen vertical columns. Neurons of the lower division of the central body have been classified into five types of tangential neuron (TL1–TL5) and two types of columnar neuron (CL1, CL2). TL1–TL4 neurons ramify in specific layers in the lower division of the central body and in the lateral triangle (TL1, TL2 neurons), the median olive (TL3 neurons), or the dorsal shell (TL4 neurons) of the lateral accessory lobe. TL5 neurons ramify in the protocerebral bridge, in the lateral accessory lobe, and in all layers of the lower division of the central body. The two types of columnar neurons have arborizations in the protocerebral bridge and in the lower division of the central body and project to the lateral triangle of the lateral accessory lobe (CL1 neurons) or to the lower subunit of the nodulus (CL2 neurons). Possible functional implications for the processing of neuronal information in the central complex are discussed.

Key words: Nervous system, insect – Central body – Protocerebrum – Golgi impregnation – Lucifer yellow – *Schistocerca gregaria* (Insecta)

Introduction

The central complex (CX) comprises a group of interconnected neuropils in the center of the insect brain. Its two major subdivisions are the protocerebral bridge (PB) and the central body (CB). The CB is further subdivided into an upper (CBU) and a lower (CBL) division and a pair of globular noduli (Williams 1975; Homberg 1991). Although the terminology for the CX and its subunits varies greatly for different insect species, the subdivisions described above appear to be present in all insects studied so far (bees: Mobbs 1985; Homberg 1985; flies: Power 1943; Strausfeld 1976; Hanesch et al. 1989; locusts: Williams 1975; Homberg 1991; for a review, see Homberg 1987).

As shown in flies, locusts, and other insect species, the CX is organized into fronto-horizontal layers intersected by eight or sixteen vertical columns (Williams 1975; Strausfeld 1976; Hanesch et al. 1989; Homberg 1991; Wegerhoff and Breidbach 1992; Vitzthum et al. 1996). This arrangement corresponds to two major classes of interneurons found in the CX, viz., tangential neurons and columnar neurons. Tangential neurons (large-field neurons in flies) innervate specific layers of a CX subunit. Columnar neurons (small-field neurons in flies) connect single columns of the same layer or of different CX layers and provide precise right-left connections within the CX (Hanesch et al. 1989; Homberg 1991; Vitzthum et al. 1996). Both classes of neurons often have additional arborizations in the surrounding protocerebrum, most frequently in the lateral accessory lobes (LALs; ventral bodies in flies). The topographic organization of the CX, together with its central position in the brain, suggests that it is involved in the integration of signals from the right and left brain hemispheres.

Several lines of evidence indicate that the CX has a function in motor control and visually guided behavior. Lesions and electrical stimulation of the CX in crickets affect respiration, stridulation, escape, and walking behavior (Huber 1960a, b; Otto 1971). *Drosophila melanogaster* mutants with structural defects in the CX are impaired in several aspects of walking and flight behavior (Strauss et al. 1992; Strauss and Heisenberg 1993; Ilius et al. 1994); flight-correlated neuronal activity in CX neurons of locusts (Homberg 1994a) also indicates a function of the CX in motor control. On the other hand,

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activity-labeling experiments with 2-deoxyglucose in *D. melanogaster* (Bausenwein et al. 1994) and single-cell recordings in bees (Homberg 1985; Milde 1988), crickets (Schildberger 1982), and locusts (Homberg 1994a) suggest a role of the CX in visual integration. Recently, we have shown that neurons in the CBL of the locust *Schistocerca gregaria* are sensitive to polarized light (Müller and Homberg 1994; Homberg and Müller 1995). This indicates a possible function of the CBL in the orientation of the locust with respect to the polarization pattern of the sky.

Other than a detailed study in *D. melanogaster* (Hanesch et al. 1989), comprehensive catalogs of neuronal cell types in the CX do not exist. To provide a background for future investigations into the role of the CX in polarized-light detection, we have studied the anatomical organization of the CBL of the locust in detail, special attention being paid to the layering and columnar organization of the CBL, the morphology of neuronal cell types, and their projections outside the CBL. Parts of this study have been published in abstract form (Müller et al. 1995, 1996).

Materials and methods

Animals

Sexually mature male and female locusts, *Schistocerca gregaria*, were obtained from crowded laboratory cultures at the University of Regensburg. Animals were reared under light-dark cycles of 12:12 h, at a temperature of 34° C during the light phase and 27° C during the dark phase. Animals were anesthetized by chilling to 4° C prior to dissection.

Bodian staining

For general neuropil staining, Bodian's silver-proteinate technique was used. Dissected brains were fixed for 3–4 h in a mixture consisting of 10% formalin, 5% glacial acetic acid and 85% ethanol, dehydrated, embedded in Paraplast Plus (Sigma, Deisenhofen, Germany), sectioned at a thickness of 10 μm, and stained according to the Bodian-protargol procedure (Bodian 1936). Briefly, deparaffinized sections were incubated for 16–20 h at 60° C in 2% aqueous Argent Proteinate solution (Prolabo, Paris, France), containing 7.5 g copper mesh/250 ml solution. Reduction, gold toning, and differentiation were performed as described (Bodian 1936). Sections were finally dehydrated and mounted in Entellan (Merck, Darmstadt, Germany).

Golgi staining

A combination of the Golgi-Colonnier method (Colonnier 1964) and Golgi-rapid method (Strausfeld 1980) was used for the impregnation of single neurons. Brains of adult locusts, 10–14 days after adult emergence, were dissected out of the head capsule in an aqueous solution of 2.5% potassium dichromate, 1.3% sucrose (PDS). Brains were immersed for 5 days at 4° C in 4 parts PDS and 1 part 25% glutaraldehyde, washed several times in PDS, incubated at 4° C in 9 parts PDS/1 part 1% osmium tetroxide for a further 5 days, rinsed and immersed for 24 h at 4° C in an aqueous solution of 0.75% silver nitrate, and finally rinsed in distilled water, dehydrated in ethanol, and embedded in Epon (Serva, Heidelberg, Germany). Frontal, sagittal, and horizontal sections were cut at a thickness of 30 μm with a sliding microtome (Reichert-Jung, Wien, Austria) and coverslipped in Epon. Neurons of the CBL were stained in 59 out of 236 Golgi-impregnated brains and were subsequently used for evaluation.

Lucifer-yellow staining

Individual neurons of the CBL were injected with the fluorescent dye Lucifer yellow via glass micropipettes. Adult locusts, 2–4 weeks old, were used for the injections. The electrodes were pulled from 1.5 mm glass capillaries (Hilgenberg, Malsfeld, Germany) and had resistances of 150–200 MΩ in the tissue. Electrode tips were filled with 4% Lucifer yellow (Fluca, Neu-Ulm, Germany, and Sigma) diluted either in distilled water or in 0.5 M LiCl, and shafts were filled with 1.0 M LiCl. The head capsule of immobilized animals was opened frontally to expose the brain. The brain was stabilized with a steel platform underneath and a steel ring on top. After removal of the ganglionic sheet in the region of the median ocellar nerve, a microelectrode was inserted, and single cells were impaled at a depth of 130–250 μm relative to the brain surface. Lucifer yellow was injected iontophoretically with a negative DC current (1 nA) for 3–7 min, following which, brains were dissected out, fixed for at least 1 h in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), dehydrated in ethanol, cleared in methyl salicylate, and examined with a fluorescence microscope for successful staining. For permanent visualization and detailed analysis of injected neurons, brains were rehydrated, embedded in gelatin/albumin, and sectioned at a thickness of 30 μm with a Vibratome (Technical Products, St. Louis, Mo., USA) in the frontal plane. Free-floating sections were stained with an anti-Lucifer-yellow antiserum (Molecular Probes, Eugene, Ore., USA) according to the peroxidase-antiperoxidase technique of Sternberger (1979) as described by Homberg (1991). The anti-Lucifer serum was diluted 1:1000 in 0.1 M TRIS HCl, 0.3 M NaCl (pH 7.4) containing 1% normal goat serum and 0.5% Triton X-100 and was applied to the sections for at least 20 h at room temperature. Goat anti-rabbit serum (Sigma) was used at a dilution of 1:40 and rabbit peroxidase-antiperoxidase (Dako, Hamburg, Germany) at 1:300. Following the diaminobenzidine reaction, sections were rinsed in phosphate buffer, mounted on gelatin-coated glass slides, dehydrated, cleared in xylene, and embedded in Entellan (Merck) under glass coverslips. Data are based on 37 successfully injected neurons of the CBL.

Reconstruction of neurons

For the reconstruction of Golgi- and Lucifer-stained neurons, a Zeiss Standard microscope (Zeiss, Oberkochen, Germany) with a camera lucida attachment was used. Photomicrographs were taken on 35 mm Agfapan 25 film (Agfa-Gevaert, Leverkusen, Germany) with a Zeiss Axiophot and a Zeiss Axioplan microscope. The orientation of brain sections and figures is given with respect to the longitudinal body axis of the locusts. The nomenclature of brain structures follows Williams (1975) and Homberg (1987).

Results

Gross anatomy of the CX and associated neuropils

The anatomical organization of the CX of the locust, *S. gregaria* is summarized in Figs. 1, 2. The CX occupies the center of the protocerebrum and lies between the pedunculi (lateral demarcation) and the β-lobes (ventral demarcation) of the mushroom bodies (Fig. 1A). The PB

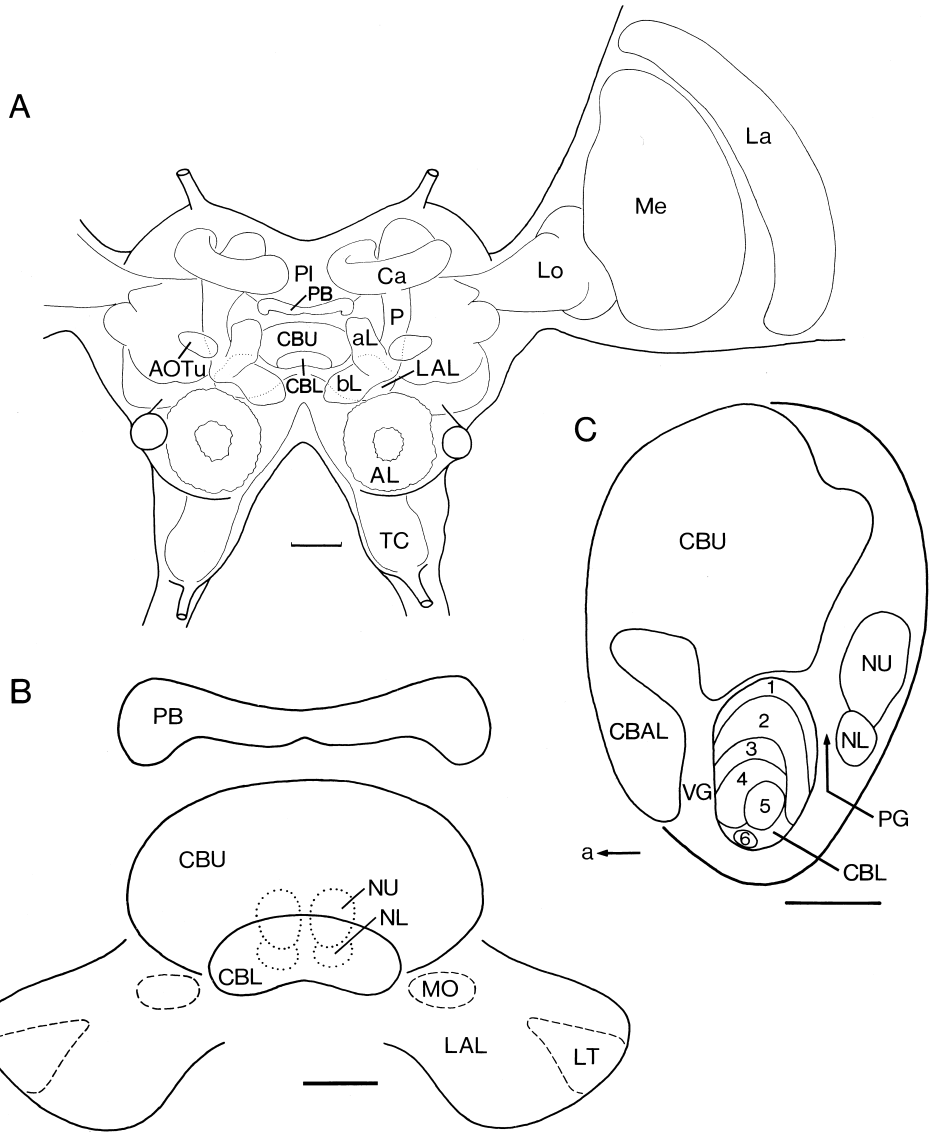


Fig. 1A–C. A Frontal diagram of the brain of *Schistocerca gregaria*. The protocerebral bridge (*PB*) and the upper and lower divisions (*CBU*, *CBL*) of the central body are situated in the center of the brain, between the α- and β-lobes of the mushroom bodies (*aL*, *bL*). The lateral accessory lobes (*LAL*, *dotted lines*) lie posterior to the β-lobes. **B** Frontal diagram of central-complex neuropils and the lateral accessory lobes (*LAL*). *Dotted lines* delineate the noduli posterior to the *CBU* and *CBL*. Each nodulus consists of an upper (*NU*) and a lower (*NL*) subunit. The median olive (*MO*) and the lateral triangle (*LT*), two distinct neuropil areas within the *LAL*, are shown in *dashed outline*. **C** Sagittal diagram of the central body. The six layers of the CBL are numbered *1–6* from dorsal to ventral. *a*, Anterior; *AL*, antennal lobe; *AOTu*, anterior optic tubercle; *Ca*, calyx of the mushroom body; *CBAL*, anterior lip of the central body; *La* lamina; *Lo*, lobula; *Me*, medulla; *P*, pedunculus of the mushroom body; *PG*, posterior groove; *PI*, pars intercerebralis; *TC*, tritocerebrum; *VG*, ventral groove. *Bars:* **A** 200 μm; **B** 100 μm; **C** 50 μm

is situated in the superior protocerebrum below the pars intercerebralis (Figs. 1, 2A, B). It has a rod-like shape with a transverse longitudinal axis and postero-ventrally curved ends. The CB lies below the PB. Its upper (CBU) and lower divisions (CBL) are kidney-shaped, with the smaller CBL lying in the ventral concavity of the CBU (Figs. 1, 2A; the schematic sagittal view of the CX in Fig. 1C shows that the anterior lip of the CBU extends in front of the CBL). The paired noduli lie at the posterior ventral edge of the CB (Figs. 1B, C, 2B) and each consists of a larger upper subunit and a smaller lower subunit. Ventro-laterally, the CB is connected bilaterally via an isthmus of neuropil (Williams 1972; Homberg 1987, 1991) to the LALs (Figs. 1B, 2A, B, D), which lie posterior to the β-lobes of the mushroom bodies. Two distinct neuropil areas can be identified within the LAL: the median olive, near the lateral edge of the CBL, and the lateral triangle in the distal LAL, close to the antennal lobe (Figs. 1B, 2D). Both areas are flanked by fibers of the isthmus tract connecting the CB and LAL.

Stratification of the CBL

A comparision of the morphologies of single neurons with Bodian preparations revealed that the CBL was subdivided into six layers (Figs. 1C, 2A, C), numbered 1–6 from dorsal to ventral. Layer 1 formed the narrow dorsalmost shell of the CBL and continued posteriorly below the level of the noduli. Layer 2 was approximately twice as wide as layer 1. It followed the course of layer 1 but posteriorly extended further into the ventral hemisphere of the CBL. Therefore, nearly the entire posterior third of the CBL was occupied by layers 1 and 2. Layers 3 and 4 lay underneath layer 2 and were restricted to the anterior two-thirds of the CBL. Layer 5 had a circular cross-section in the sagittal plane; it was covered antero-dorsally by layer 4 and posteriorly by layer 2. Layer 6 was the ventralmost layer and lay below layers 4 and 5 at the antero-ventral margin of the CBL. It was the smallest layer and, like layer 5, was circular in cross-section.

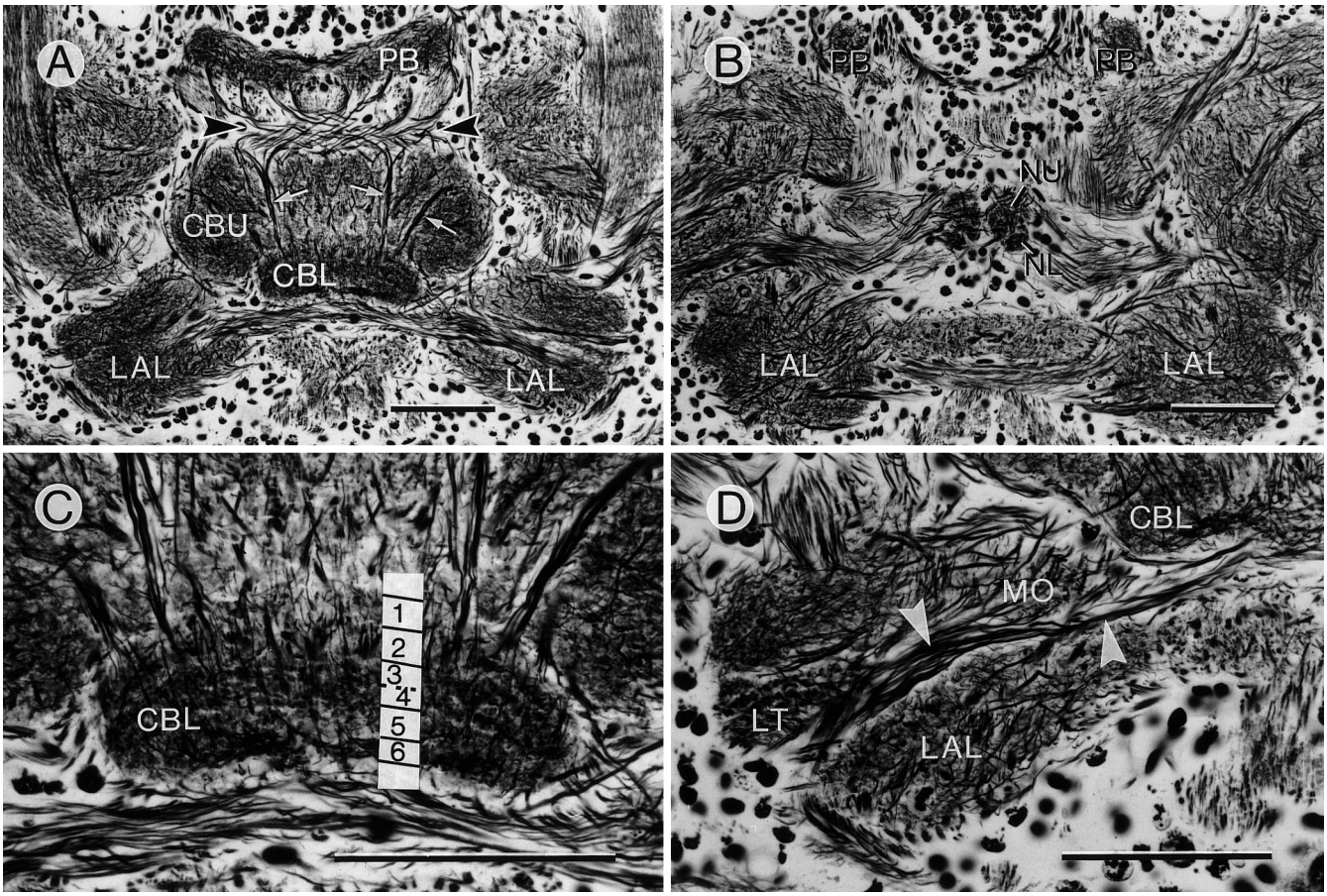


Fig. 2A–D. Bodian-stained brain sections. **A**, **B** Anterior (**A**) and posterior (**B**) frontal section through the central complex and the lateral accessory lobes (*LAL*). The sections are slightly tilted, so that the protocerebral bridge (*PB*) and the lower division of the central body (*CBL*) appear in the same section (**A**). In **A**, columnar fibers connect the *PB* and the central body through the posterior chiasma (*arrowheads*). Many of these fibers penetrate layer III of the upper division of the central body (*CBU*) via the posterior vertical bundles (*arrows*). The section in **B** is about 40 µm posterior from the section in **A**. It shows the upper (*NU*) and the lower subunits (*NL*) of the noduli and the postero-ventrally curved endings of the *PB*. **C** At higher magnification, six layers can be distinguished in the *CBL*, numbered *1–6* from dorsal to ventral. **D** Section through the lateral accessory lobe (*LAL*), at the level of the median olive (*MO*) and the lateral triangle (*LT*). Both areas are flanked by fibers of the isthmus tract (*arrowheads*), which connects the *LAL* to the central body. *Bars:* 100 µm

Neurons of the CBL

Five types of tangential neuron (termed TL1–TL5, Figs. 3–8) and two types of columnar neuron (CL1, CL2, Figs. 10–12) with arborizations in the CBL were found. Tangential neurons innervated specific layers of the CBL and had a second field of arborization in the LAL. The five types of TL neuron differed with respect to the layer that they innervated in the CBL, the arborization pattern in the LAL, and the position of the cell body. The columnar neurons of the CBL ramified in the PB, in the CBL, and in the LAL (CL1 neurons) or in the noduli (CL2 neurons). Unlike the tangential neurons that innervated the CBL in its whole lateral extension, the columnar neurons had arborizations in single columns that extended through several layers.

Tangential neurons of the CBL

The first type of tangential neuron (TL1 neurons, Fig. 3) was found in six Lucifer preparations and in one Golgi preparation. The cell bodies of TL1 neurons lay in the ventro-median protocerebrum. Their primary neurites ran between the antennal mechanosensory and motor center and the LAL and entered the LAL postero-ventrally to the lateral triangle. The neurons had dense arborizations with fine endings throughout the lateral triangle (Fig. 3A, C, E). In some preparations, a few fine processes extended beyond the boundaries of the lateral triangle (Fig. 3A). The axons projected to the CB within the isthmus tract. They entered the posterior groove between the noduli and the CBL (Fig. 1C) and continued along the dorso-posterior surface of the CBL. The axons gave off sidebranches into the CBL and densely innervated layers 2–6 (Fig. 3A–D), whereas layer 1 was omitted. In some preparations, layer 6 also seemed to be omitted (Fig. 3D). The nerve endings in the CBL were beaded and larger than those in the lateral triangle (Fig. 3D, E).

TL2 neurons (Figs. 4, 5) had cell bodies in the inferior median protocerebrum, anterior to the TL1 cell bod-

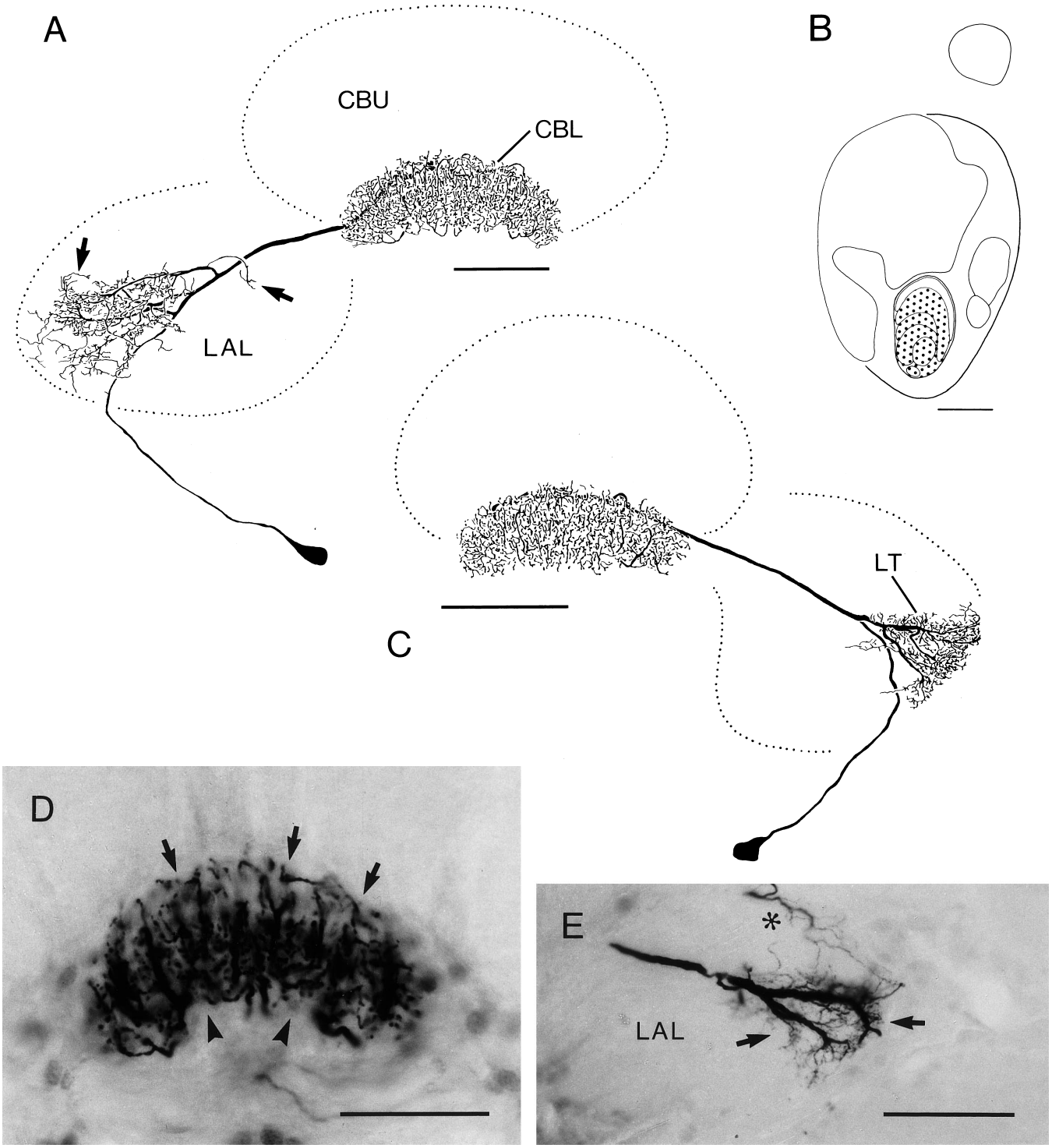


Fig. 3A–E. TL1 neurons. **A**, **C** Reconstructions of Lucifer-filled single cells from two preparations. Note the dense ramifications of these neurons in the lower division of the central body (*CBL*) and in the lateral triangle (*LT*) of the lateral accessory lobe (*LAL*). Occasionally, processes extend beyond the boundaries of the LT (*arrows* in **A**). **B** Sagittal projection of the arborization area of the two TL1 neurons (*dotted area*) in the CBL. **D**, **E** Arborizations of two different Lucifer-stained neurons in the CBL (**D**) and in the lateral triangle (**E**, *arrows*) of the *LAL*. The neuron in **D** does not invade layer 1 (*arrows*) or layer 6 (*arrowheads*) of the CBL. In **E**, a second neuron (*asterisk*) is stained faintly. *CBU,* Upper division of the central body. *Bars:* **A**, **C**–**E** 100 μm; **B** 50 μm

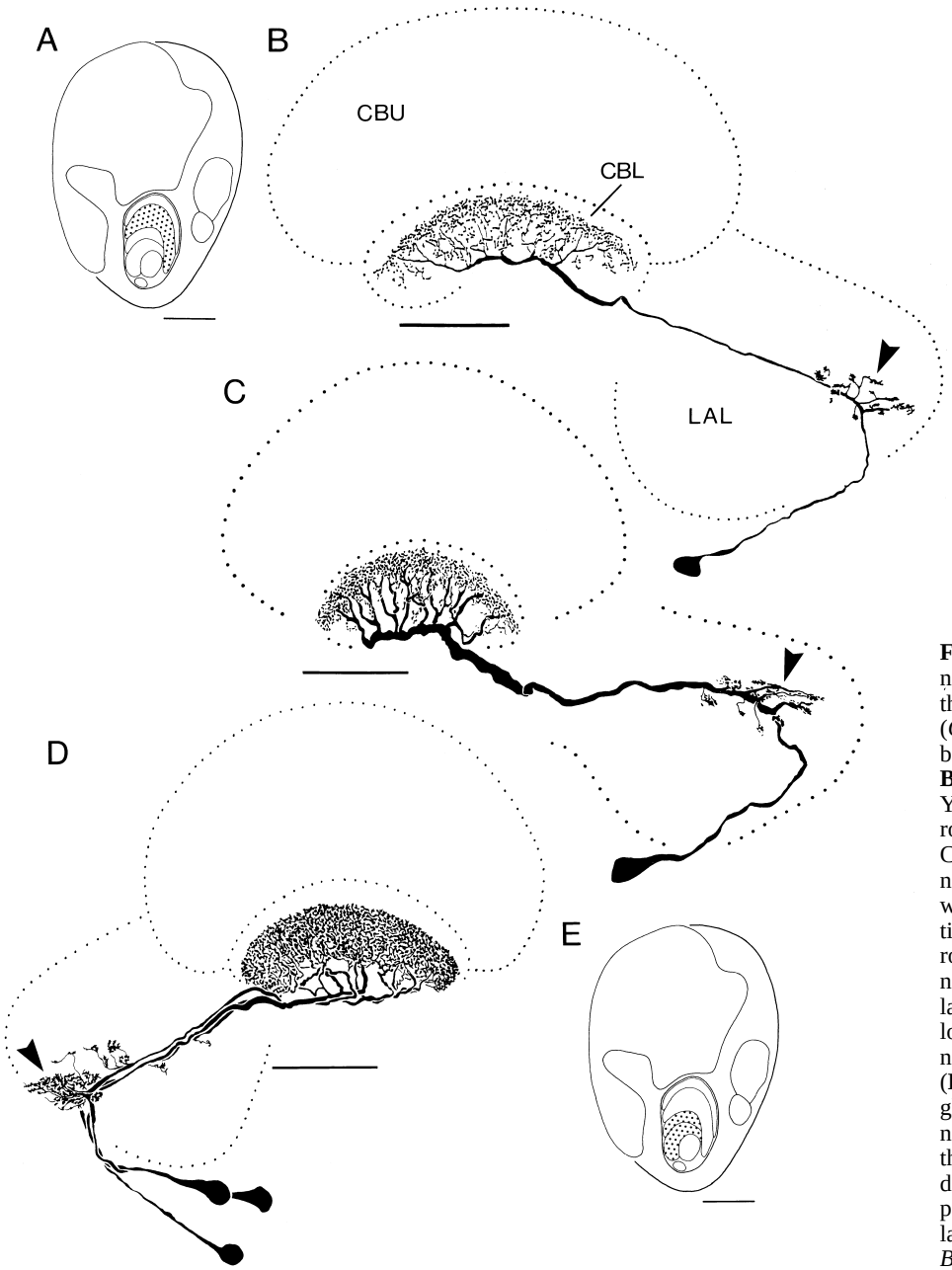


Fig. 4A–E. TL2 neurons. **A**–**C** TL2 neurons with arborizations in layer 2 of the lower division of the central body (*CBL*). **A** Sagittal projection of the arborizations in the CBL (*dotted area*). **B**, **C** Reconstructions of two Lucifer-Yellow-injected neurons. **D**, **E** TL2 neurons innervating layers 3 and 4 of the CBL. **D** Reconstruction of three TL2 neurons of similar morphology filled with Lucifer yellow. **E** Sagittal projection of the arborizations of these neurons in the CBL (*dotted area*). All TL2 neurons innervate small areas within the lateral triangle of the lateral accessory lobe (*arrowheads* in **B**–**D**). In the two neurons innervating layer 2 of the CBL (**B**, **C**), ramifications in the lateral triangle are more medial, whereas in the neurons invading layers 3 and 4 (**D**) of the CBL, arborizations are concentrated distally in the lateral triangle. *CBU*, Upper division of the central body; *LAL*, lateral accessory lobe.
Bars: **A**, **E** 50 µm; **B**–**D** 100 µm

ies. Their primary neurites ran dorsally along the antennal lobe into the LAL close to the lateral triangle. In contrast to the TL1 neurons, TL2 neurons arborized only in small subfields of the lateral triangle. The nerve endings showed a swollen irregular shape (Fig. 5A, D, E). At high magnification, these swollen endings appeared to consist of dense tangles of very fine processes. From the lateral triangle, the main fibers of TL2 cells ran through the isthmus tract to the CB and, as they continued along the ventral concavity of the CBL, gave off branches into the CBL. TL2 neurons ramified in specific layers of the CBL. Neurons with arborizations in layer 2 were encountered most frequently (n=16, Figs. 4A–C, 5A–E). The branches of these neurons had a fan-like distribution and bore numerous beaded endings (Fig. 5B, C). Another subtype of TL2 neurons arborized in layers 3 and 4 (n=2, Fig. 4D, E). The nerve endings of these neurons in the CBL were also beaded. TL2 neurons with arborizations in layers 3 and 4 seemed to innervate more lateral areas of the lateral triangle, whereas TL2 neurons with arborizations in layer 2 of the CBL ramified more medially within the lateral triangle (compare Fig. 4B–D). Tangential neurons with arborizations in layers 4 and 5 were also found (n=4, Fig. 5F, G), but ramifications in the lateral triangle could be detected in only one of these preparations.

The morphology of TL3 neurons (Figs. 6, 7A, B) was similar to that of TL2 cells with respect to cell-body position and the course of the axon. In contrast to TL2 neurons, however, TL3 neurons did not innervate the lateral

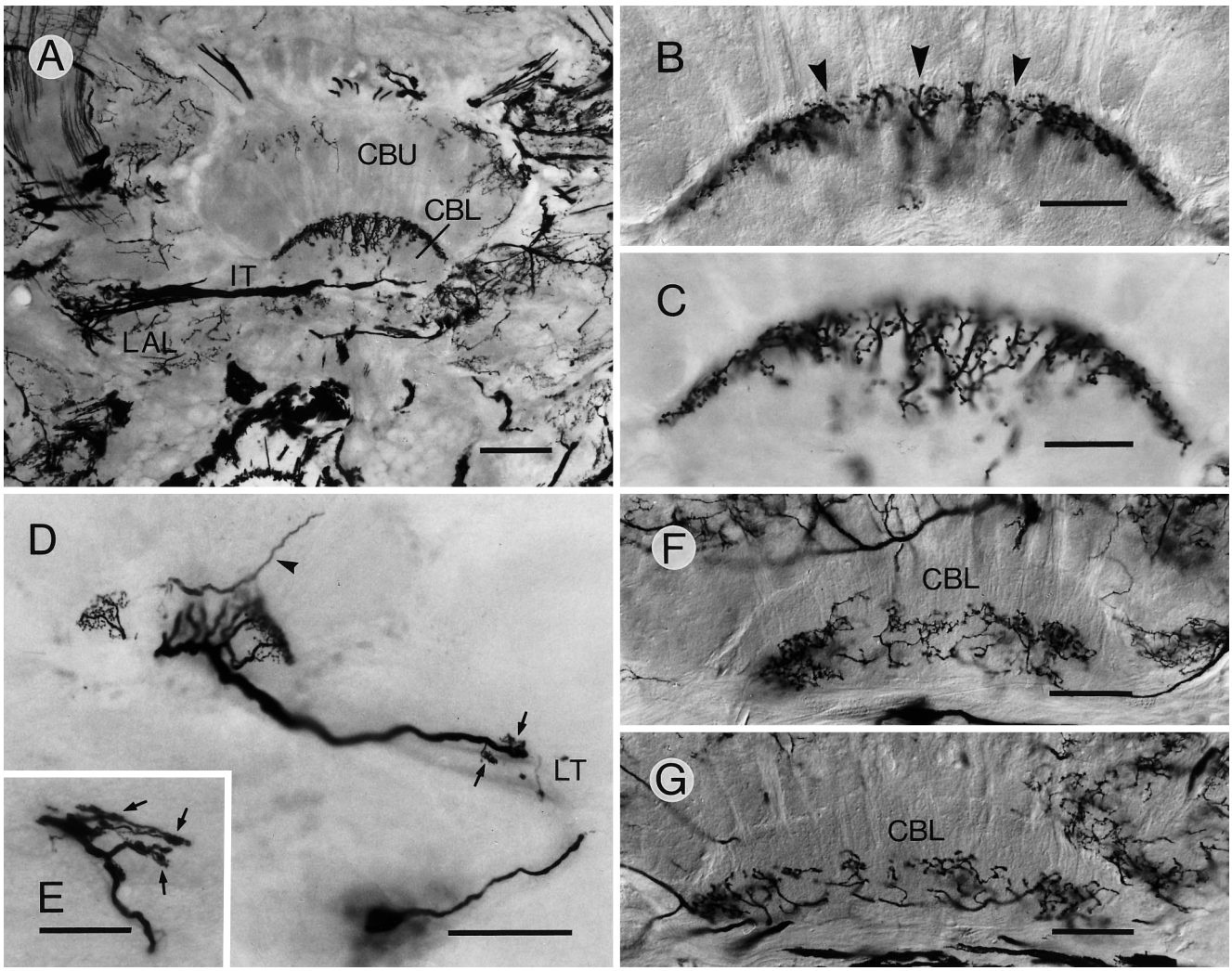


Fig. 5A–G. TL2 neurons. **A**–**C** Frontal section of a Golgi-impregnated brain, showing several TL2 neurons with arborizations in layer 2 of the lower division of the central body (*CBL*). **B** Differential interference contrast shows that the narrow layer 1 of the CBL (*arrowheads*) is not innervated by TL2 neurons. In **C**, the beaded nerve endings in the CBL are visible. **D**, **E** Consecutive frontal sections showing a Lucifer-filled TL2 neuron with ramifications in layer 2 of the CBL. This neuron is reconstructed in Fig. 4C. **E** Shows the ramifications in the lateral triangle at higher magnification. Several large swellings (*arrows* in **D** and **E**) in the lateral triangle (*LT*) appear to consist of dense tangles of very fine processes. *Arrowhead* in **D** points to a weakly stained columnar fiber of the central body. **F**, **G** Tangential neurons with arborizations in layers 4 and 5 of the *CBL*. *CBU*, Upper division of the central body; *IT*, isthmus tract; *LAL*, lateral accessory lobe. *Bars:* **A**, **D** 100 μm; **B**, **C**, **E–G** 50 μm

triangle but instead ramified in the median olive (Figs. 6A, B, 7A). Arborizations in the median olive bore large knot-like swellings that could be resolved as dense tufts of fine branches at higher magnification in the light microscope (Fig. 6B). We found TL3 neurons with arborizations in layer 5 (*n*=5, Figs. 6A, 7A, B, 8D) and in layer 2 (*n*=3; see Homberg 1994a), and neurons with arborizations in two layers, viz., layers 2 and 6 of the CBL (*n*=6, Fig. 6C–E). Layers 2, 4, and 5 were innervated in a Lucifer double-staining of two TL3 neurons. The nerve endings of TL3 neurons in the CBL were beaded like those of TL2 cells (Fig. 6C, D).

TL1, TL2, and TL3 neurons were never observed to arborize in layer 1 of the CBL. This layer appeared to be exclusively innervated by TL4 and TL5 neurons. TL4 neurons (Figs. 7C, D, 8A–D) were only stained in Golgi preparations (*n*=14). Compared with the other tangential cells, their axons were relatively small. The cell bodies of TL4 neurons occurred together with TL2 and TL3 neurons or lay more laterally between the distal edge of the LAL and the antennal lobe (Fig. 7C). Their small axons passed through the isthmus tract to the CBL. Close to the median olive, they gave off sidebranches that ramified with fine endings in the anterior dorsal shell of the LAL (Fig. 7C). The axons ran along the ventral edge of the CBL. Near the midline of the brain, they turned dorsally and ramified in an umbrella-like fashion along the posterior edge of the CBL. Fine beaded branches extended throughout layer 1 (Figs. 7C, 8A, B).

The fifth type of tangential neuron (TL5 neurons, Figs. 7E, F, 8E–G), found in three Lucifer preparations, differed substantially from the other TL neurons. The

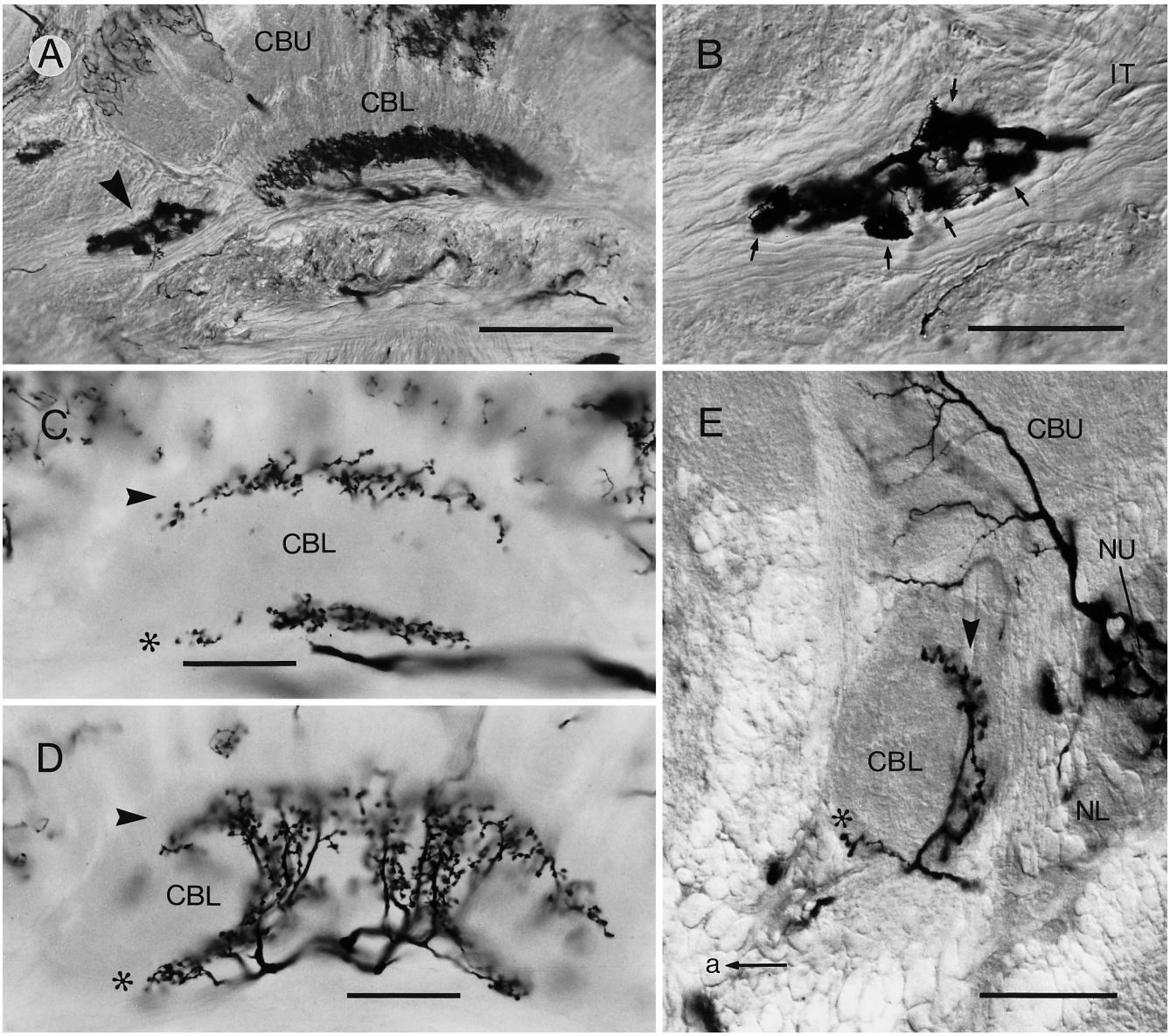


Fig. 6A–E. TL3 neurons. **A** Frontal section of a Golgi-impregnated brain. Two TL3 neurons with arborizations in layer 5 of the lower division of the central body (*CBL*) are stained. The neurons arborize in the median olive (*arrowhead*) of the LAL. **B** The ramifications in the median olive bear large knot-like swellings (*arrows*) that consist of dense tufts of fine branches. **C**, **D** Consecutive frontal sections of a Golgi-impregnated brain. Two TL3 neurons with arborizations in layer 2 (*arrowhead*) and layer 6 (*asterisk*) of the CBL are stained. The arborizations have numerous beaded endings. **E** Sagittal sections of a Golgi-impregnated brain. A TL3 neuron with ramifications in layer 2 (*arrowhead*) and layer 6 (*asterisk*) is labeled. A tangential neuron of the upper division of the central body (*CBU*) is also stained. This neuron innervates the upper subunit of the noduli (*NU*). *a* Anterior; *CBU*, upper division of the central body; *IT*, isthmus tract. *Bars:* **A**, **C**, **D** 100 μm; **B**, **E** 50 μm

cell bodies of TL5 neurons lay in the pars intercerebralis. The neurons densely innervated the ipsilateral hemisphere of the PB with fine branches (Figs. 7F, 8G). In addition, some processes extended into the superior protocerebrum directly below the PB (Fig. 7F). From the bridge, the large main fibers passed through the w-bundles (Williams 1975) to the LAL. A second field of fine arborizations extended throughout the dorsal hemisphere of the LAL, apparently including the lateral triangle (Figs. 7F, 8F). The axons continued medially through the posterior groove and entered the CBL posteriorly. Beaded arborizations uniformly innervated all layers of the CBL. One of the stained TL5 neurons had additional ramifications in the lower subunit of the contralateral nodulus, and another had arborizations in the ipsilateral posterior optic tubercle (not shown). The morphology of the five types of TL neurons is summarized in Table 1.

In addition to the TL neurons, which innervated only the lower division of the CB, a few tangential neurons appeared to arborize both in the CBU and CBL. These neurons were incompletely stained in Golgi preparations but, in general, seemed to have sparse and diffuse processes in the CBL and CBU. One of these neurons was

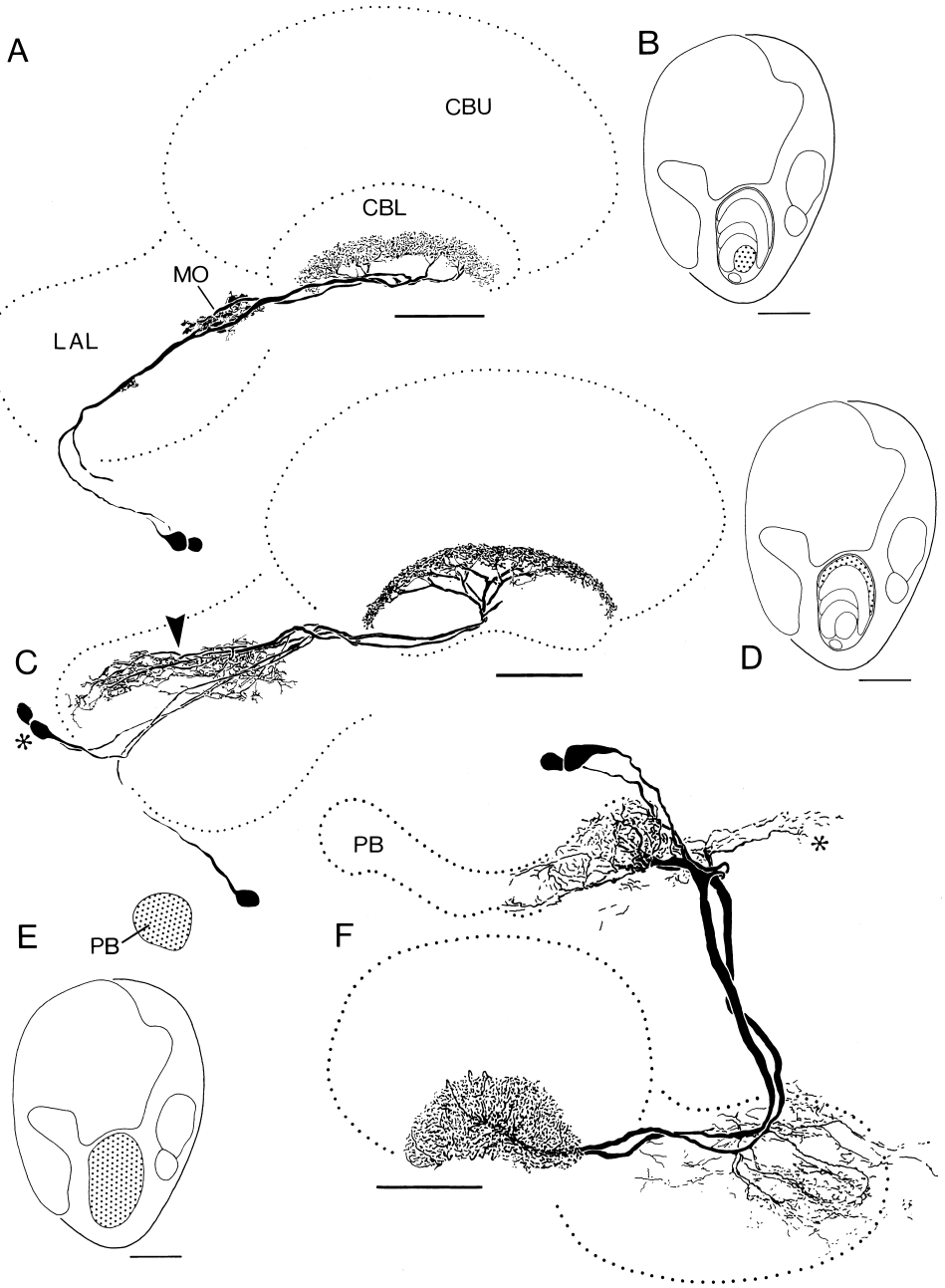


Fig. 7A–F. Frontal reconstructions and sagittal diagrams of central-complex arborizations (*dotted areas*), respectively, of TL3 neurons (**A**, **B**), TL4 neurons (**C**, **D**), and TL5 neurons (**E**, **F**). **A** Reconstruction of two TL3 neurons with arborizations in layer 5 of the lower division of the central body (*CBL*) and in the median olive (*MO*) of the lateral accessory lobe (*LAL*) from the Golgi preparation shown in Fig. 6A, B. **C** Reconstruction of three TL4 neurons from a Golgi-impregnated brain. The neurons arborize in layer 1 of the CBL and in the anterior dorsal shell of the LAL (*arrowhead*). Two of the neurons have cell bodies between the lateral face of the LAL and the antennal lobe (*asterisk*). **F** Reconstruction of two TL5 neurons from a Lucifer-yellow-injected brain. The neurons have cell bodies in the pars intercerebralis and arborize in the protocerebral bridge (*PB*), in the superior median protocerebrum (*asterisk*), in dorsal parts of the LAL, and throughout the CBL. *CBU*, Upper division of the central body. *Bars:* **A**, **C**, **F** 100 μm; **B**, **D**, **E** 50 μm

stained with Lucifer yellow (Fig. 9). The soma of this neuron lay in the pars intercerebralis. Fine ramifications invaded the superior protocerebrum, the ventro-median protocerebrum, and both LALs. An axonal fiber passed along the midline over the anterior surface of the CB and gave rise to fine processes concentrated in layers 3–6 of the CBL and layer IIa of the CBU.

Columnar neurons of the CBL

The first type of columnar neuron (CL1 neurons, Figs. 10, 11) had cell bodies in the pars intercerebralis. Small primary neurites ran to the anterior face of the PB and gave rise to arborizations within the ipsilateral hemisphere of the bridge. Ramifications were confined to laterally restricted areas, termed columns of the PB. Large diameter axons left the PB ventrally and ran through the w-, x-, y-, and z-bundles of the posterior chiasma (Williams 1975, Fig. 2A) into the CBU. CL1 fibers passed as part of the posterior vertical bundles (Williams 1972) through layer III of the CBU to the dorsal surface of the CBL (Figs. 2A, 11I, J). Before entering the CBL, the axons usually divided into two or more parallel branches that entered the CBL and into one fine axon that ran along the anterior surface of the CBL to the ventral groove (Figs. 10A–C, 12B). In the CBL, as in the PB, the neurons arborized in columnar domains. The axons continued along the ventral groove (Fig. 1C) to the contralateral LAL, ran through the isthmus tract, and termi-

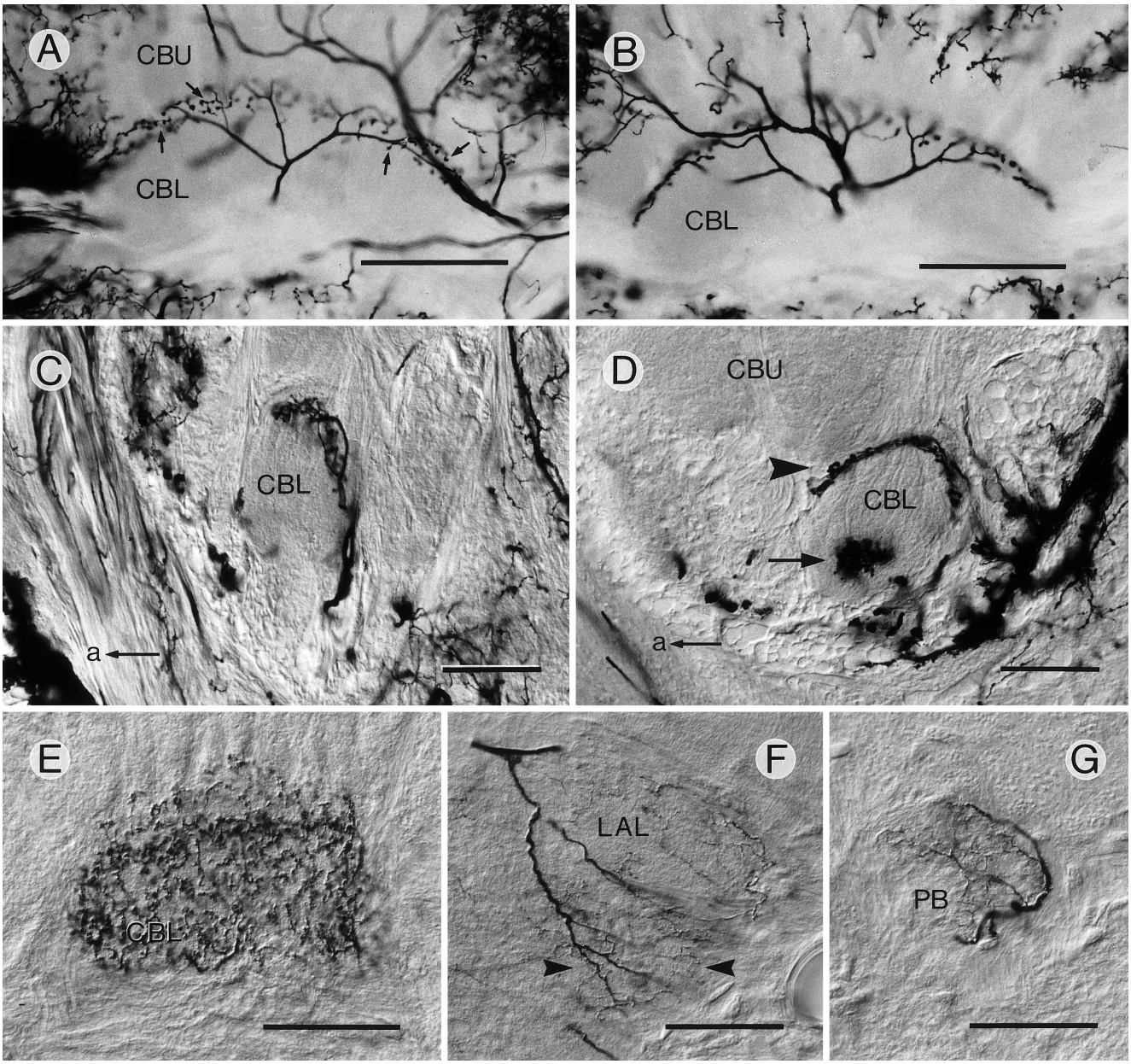


Fig. 8. A–D TL4 neurons. **E–G** TL5 neurons. **A**, **B** Frontal sections of two Golgi-impregnated brains showing arborizations of single TL4 neurons in the lower division of the central body (*CBL*). The main fiber branches in an umbrella-like fashion and innervates layer 1 of the *CBL*. *Arrows* in **A** mark some of the beaded fiber endings. **C**, **D** Sagittal sections of two Golgi-impregnated brains. **C** A TL4 neuron is labeled. **D** A TL4 cell (*arrowhead*) and a TL3 neuron with arborizations in layer 5 of the CBL (*arrow*) are stained. *a*, Anterior; *CBU*, upper division of the central body. **E–G** Frontal sections of a pair of Lucifer-yellow-injected TL5 neurons. **E** The *CBL* is densely innervated in all layers. **F** Arborizations in the lateral accessory lobe (*LAL*) are concentrated in dorsal areas and apparently include the lateral triangle (*arrowheads*). **G** Arborizations in the protocerebral bridge (*PB*). *Bars:* **A**, **B** 100 μm; **C–G** 50 μm

nated in beaded arborizations in subfields of the lateral triangle (Figs. 10A–C, 11K, L).

CL1 neurons provided precise topographical connections between columns of the PB and columns of the CBL and corresponded to the system of 64 CC1 neurons described by Williams (1975). The topographical organization of the CL1 system is schematically illustrated in Fig. 10D. Eight columns could be distinguished in each hemisphere of the PB and were numbered R1–R8 and L1–L8 from the lateral ends of the bridge to the center. Corresponding to the 16 columns in the PB, 16 columnar zones of arborization were also found in the CBL and were numbered 1–16 from right to left. Columns of the right and left hemispheres of the PB were connected through the CL1 neurons to alternating columns of the CBL, thereby mapping columns R1–R8 of the PB to odd numbered columns of the CBL, and columns L1–L8 to even numbered columns of the CBL. Irrespective of the

Table 1. Branching patterns of tangential neurons of the locust CBL (*CBL*, lower division of the central body; *LAL*, lateral accessory lobe; *NL*, lower subunit of the nodulus; *PB*, protocerebral bridge; *POTu*, posterior optic tubercle)

Tangential neurons	Cell-body location	Arborization areas outside the CBL	Layers innervated within the CBL	Number of preparations
TL1	Ventro-median protocerebrum	Throughout the lateral triangle of the LAL	Layers 2–6	7
TL2	Inferior-median protocerebrum	Small areas in the lateral triangle of the LAL	Layer 2 Layers 3 and 4 Layers 4 and 5	16 2 1
TL3	Inferior-median protocerebrum	Median olive of the LAL	Layer 5 Layer 2 Layers 2 and 6	5 3 6
TL4	Inferior-median or inferior-lateral protocerebrum	Dorsal shell of the LAL	Layer 1	14
TL5	Pars intercerebralis	Dorsal parts of the LAL; ipsilateral hemisphere of the PB (NL, POTu)	Layers 1–6	3

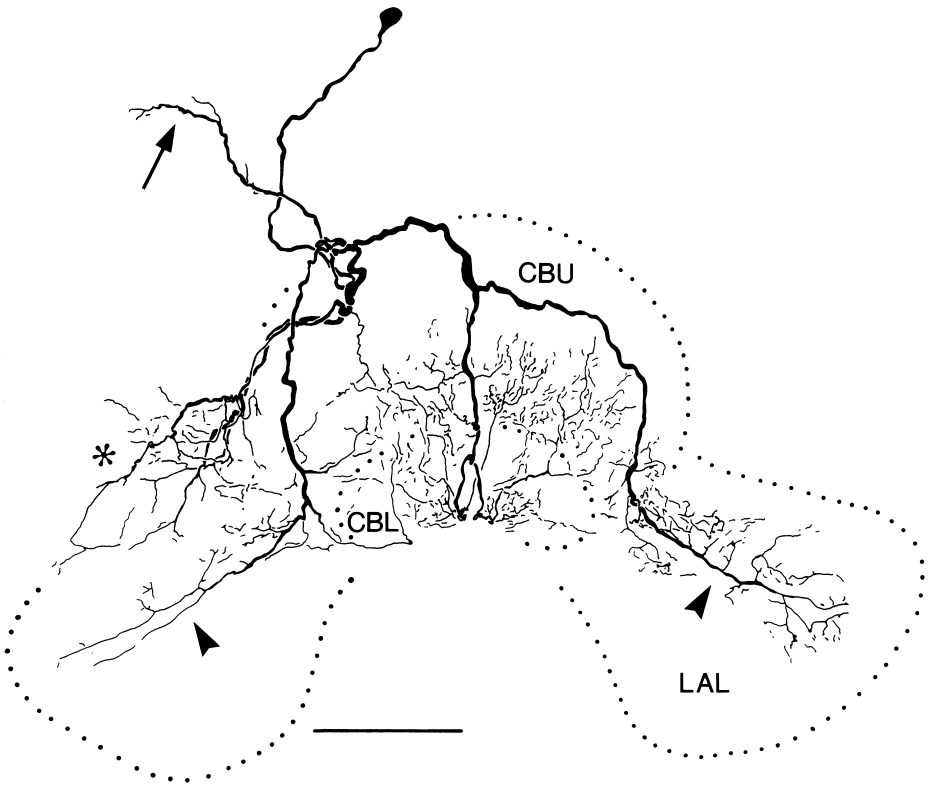


Fig. 9. Frontal reconstruction of a Lucifer-yellow-filled tangential neuron with ramifications in the lower (*CBL*) and the upper (*CBU*) division of the central body. The neuron has additional arborizations in the superior protocerebrum (*arrow*), in the ventro-median protocerebrum (*asterisk*), and in both lateral accessory lobes (*LAL*, *arrowheads*). *Bar:* 100 μm

column innervated in the CBL, all CL1 neurons projected to the contralateral lateral triangle. Fibers crossed the midline either in the posterior chiasma (neurons R5–8 and L5–8) or in the ventral groove (R1–4, L1–4). CL1 neurons of each column were stained at least once in the Lucifer and Golgi preparations, except for neurons connecting the PB columns R5, R8, and L7 to the CBL. In some preparations, more than one neuron with an identical fiber course was stained (Fig. 11I, J), consistent with the four CL1 neurons per column reported by Williams (1975).

In contrast to the ethyl-gallate preparations of Williams (1975), Lucifer-yellow staining and Golgi impregnation of single cells allowed a more detailed investigation of the arborization fields of the CL1 neurons in the various neuropils. An examination of all preparations in which CL1 neurons were stained (n=34) revealed that the arborizations in the PB and in the CBL were not

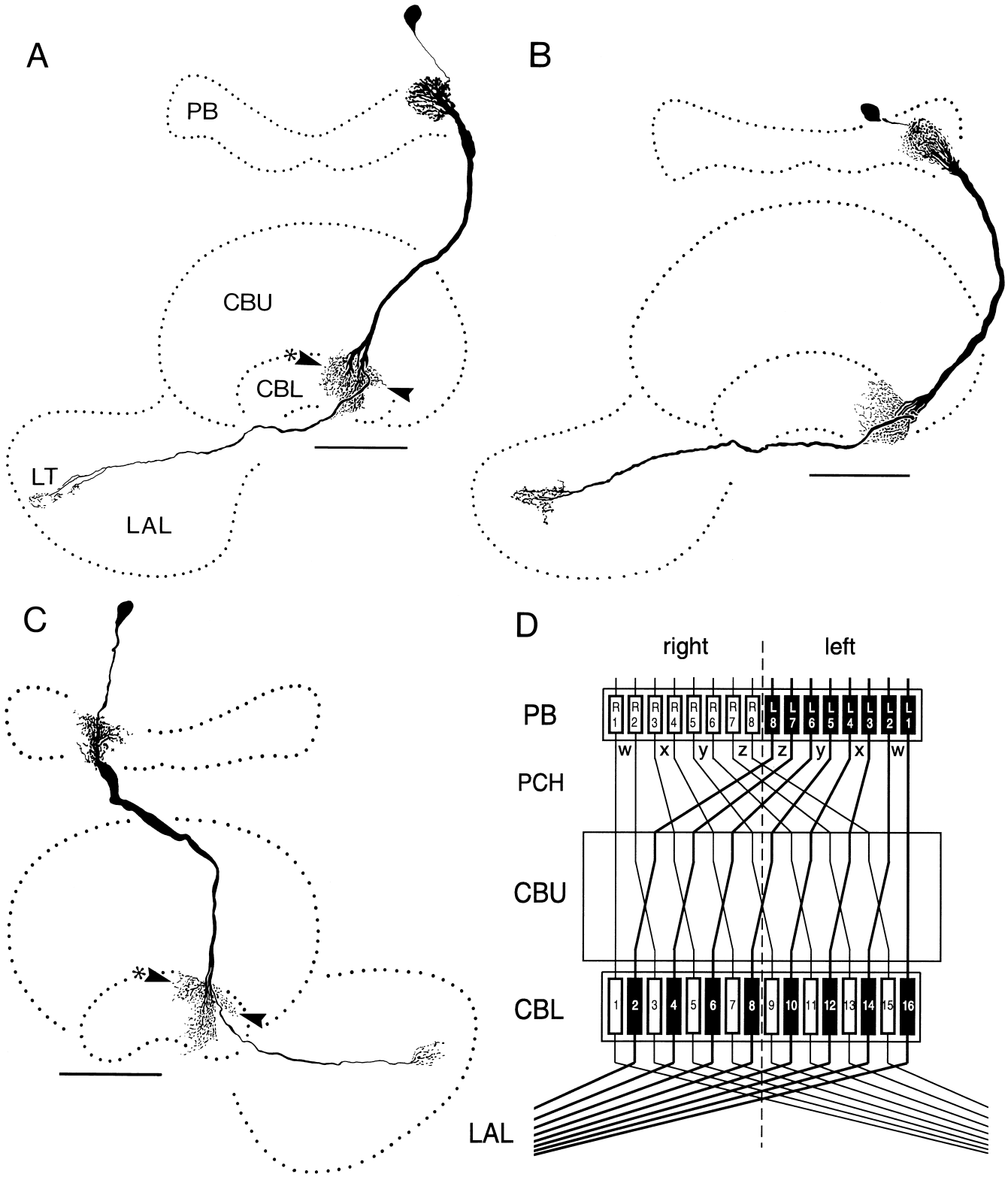
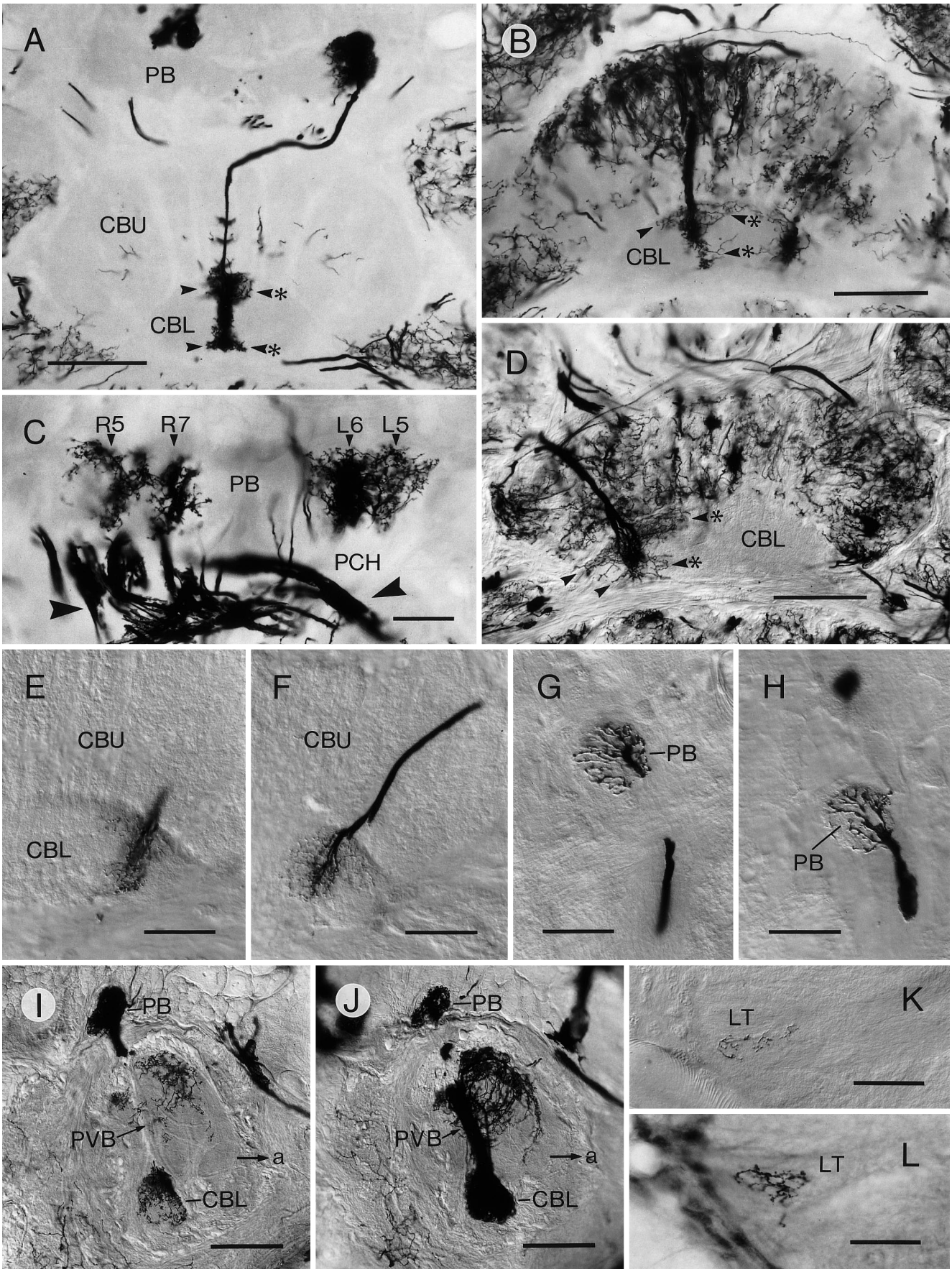


Fig. 10A–D. CL1 neurons. **A**–**C** Frontal reconstructions of three CL1 neurons from Lucifer-yellow-injected brains. The neurons have cell bodies in the pars intercerebralis, innervate columns in the ipsilateral hemisphere of the protocerebral bridge (*PB*) and in the lower division of the central body (*CBL*), and send axons to the lateral triangle (*LT*) of the contralateral lateral accessory lobe (*LAL*). *Arrowheads* in **A** and **C** indicate arborizations in dorsal CBL layers that invade neighboring columns. This lateral spread of processes is more pronounced toward the midline of the CBL (*arrowhead with asterisk*) than to the periphery. **D** Schematic diagram of the CL1-neuron system. Each column is represented by a *rectangle. White rectangles* connected by *fine lines* represent neurons arborizing in the right hemisphere of the protocerebral bridge (*PB*); *black rectangles* connected by *solid lines* represent neurons ramifying in the left *PB* hemisphere; *dashed line* indicates the midline of the brain. In the posterior chiasma (*PCH*), fibers run in the w-, x-, y-, and z-bundles to the central body. *CBU,* Upper division of the central body. *Bars:* **A**–**C** 100 μm


A
PB
CBU
CBL
B
CBL
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R7
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Fig. 11A–L

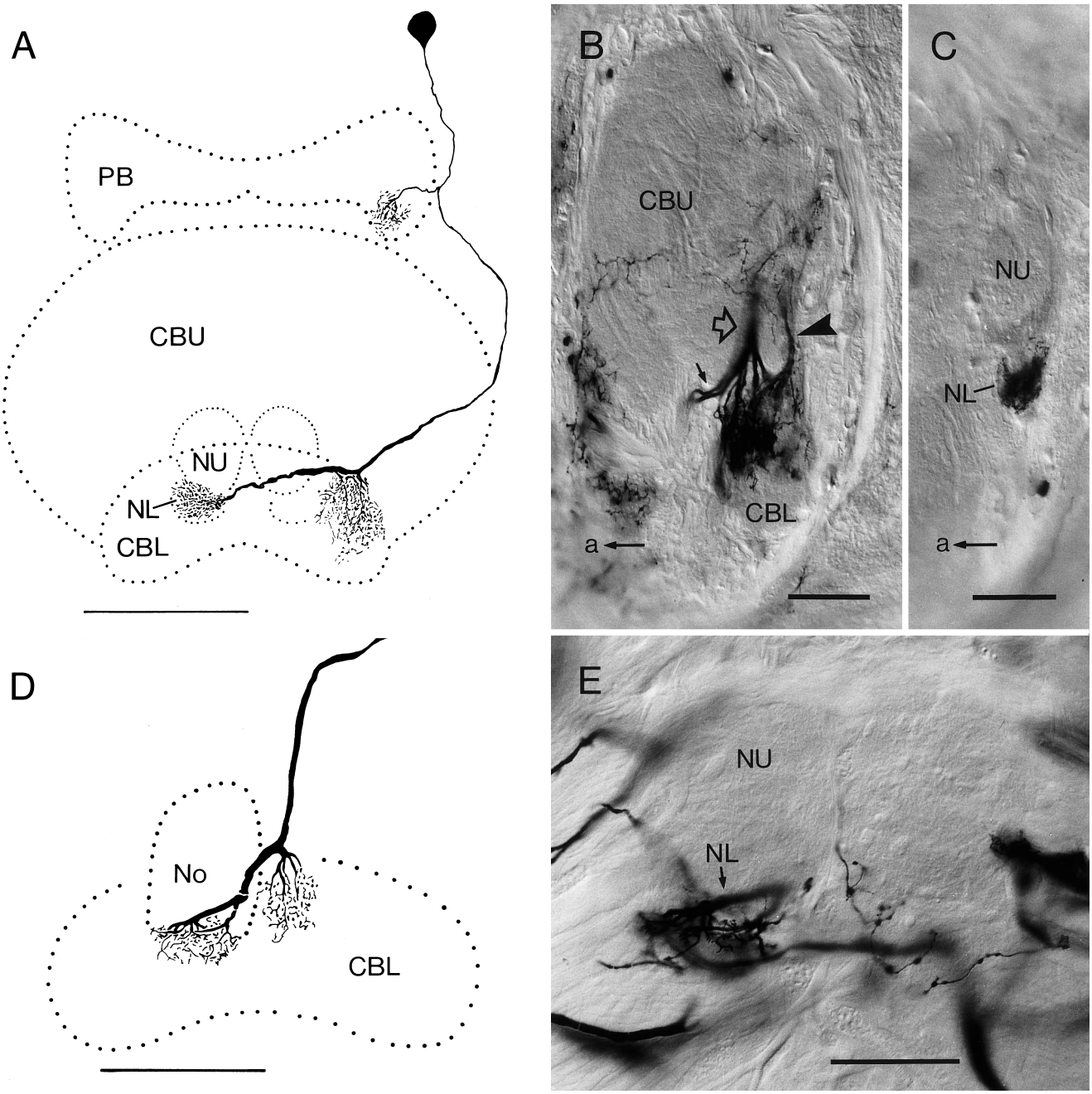

Fig. 12A–E. CL2 neurons. **A** Frontal reconstruction of a Lucifer-filled CL2 neuron. The neuron has arborizations in the protocerebral bridge (*PB*), in dorsal layers of the lower division of the central body (*CBL*), and in the lower subunit of the contralateral nodulus (*NL*). **B**, **C** Two sagittal sections of a Golgi-impregnated brain showing the arborizations of two CL1 neurons and one CL2 neuron in the *CBL* (**B**) and the arborizations of the CL2 neuron in the lower nodulus subunit (**C**, *NL*). The axon of the CL2 neuron (**B**, *arrowhead*) runs more posteriorly within the posterior vertical bundle than the axons of the CL1 cells (**B**, *open arrow*). *Arrow* in **B**, Axons of the two CL1 neurons that enter the ventral groove toward the lateral triangle. *a*, Anterior. **D** Frontal reconstruction of the ramifications of a CL2 neuron in the *CBL* and the nodulus (*No*) from a Golgi-impregnated brain. **E** Arborizations in the lower subunit of the contralateral nodulus (*NL*) from the neuron reconstructed in **D**. *CBU*, Upper division of the central body; *NU*, upper subunit of the nodulus. *Bars:* **A**, **D** 100 μm; **B**. **C**, **E** 50 μm

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Fig. 11A–L. CL1 neurons. **A**–**D** Frontal sections of Golgi-impregnated brains. **A** A CL1 neuron connecting column L5 of the protocerebral bridge (*PB*) with column 8 of the lower division of the central body (*CBL*). **B**, **D** Arborizations of CL1 neurons in the *CBL*. *Arrowheads* in **A**, **B** and **D** point to the lateral spread of arborizations into neighboring columns in layers 1, 2, and 6 of the *CBL*. These lateral projections are more pronounced toward the midline of the *CBL* (*asterisks*). **C** Ramifications of four columnar neurons in the protocerebral bridge (*PB*). Columns *R5* and *R7* are labeled in the right hemisphere of the bridge, and columns *L5* and *L6* are labeled in the left hemisphere. The arborizations of the neighboring columns *L5* and *L6* overlap, whereas the ramifications of columns *R5* and *R7* extend into column R6 in between. Several fibers in the posterior chiasma (*PCH*) are stained (*arrowheads*). **E**–**H** Frontal sections of a Lucifer-yellow-injected brain, showing the arborizations of a CL1 neuron in the *CBL* (**E**, **F**) and the *PB* (**G**, **H**). The neuron is reconstructed in Fig. 10A. **I**, **J** Consecutive sagittal sections of a Golgi-impregnated brain, showing several CL1 neurons with fibers in the same posterior vertical bundle (*PVB*). The whole cross-section of the CBL is densely innervated. *a*, Anterior. **K**, **L** Arborizations of the CL1 neurons of Fig. 10A (**K**), B (**L**) in the lateral triangle (*LT*) of the lateral accessory lobe. *CBU*, Upper division of the central body. *Bars:* **A**, **B**, **D**, **I**, **J** 100 μm; **C**, **E**–**H**, **K**, **L** 50 μm

strictly confined to individual columns but covered more than one sixteenth of the width of the PB and the CBL (i.e., the theoretical column width). In the PB, the ramifications of neighboring CL1 neurons seemed to overlap laterally by about 25%–50% (Fig. 11C). The arborization fields in the CBL were more complex. Some of the CL1 neurons arborized in all layers of the CBL (Fig. 11J), whereas others seemed to omit layer 5 and possibly also layers 3 and 4. Moreover, the lateral overlap of the arborizations between neurons of neighboring columns differed for different CBL layers. In layers 1, 2, and 6, processes invaded one or two adjacent columns on each side of the main arborization column (Figs. 10A–C, 11A, B, D–F). Usually, these lateral projections were more pronounced toward the midline of the CBL than to the sides (Figs. 10A, C, 11A, B, D). In contrast, the arborizations in layers 3, 4, and 5 were confined to single columns or extended only slightly into neighboring columns. In Golgi preparations, the arborizations of CL1 neurons in the PB and the CBL had fine endings (Fig. 11A–D, I, J), whereas in Lucifer-stained preparations, ramifications in the PB had a mixed fine-beaded appearance (Figs. 10A–C, 11G, H).

The second type of columnar neuron, the CL2 neuron, was stained in nine preparations (Fig. 12). Like CL1 neurons, CL2 neurons had somata in the pars intercerebralis and arborized in distinct columns of the PB and the CBL. We could not determine the exact size of their arborization areas in the PB and CBL, but, in the CBL, their ramifications were restricted to dorsal layers, probably layers 1–4 (Fig. 12A, B, D). The CL2 fibers connecting the PB and the CBL were smaller than those of the CL1 neurons (Fig. 12A) and ran more posteriorly within the posterior vertical bundles through the CBU (Fig. 12B). At the dorso-posterior edge of the CBL, sidebranches were given off into the CBL, and the main fiber continued into the posterior groove to the contralateral nodulus. The lower subunit of the contralateral nodulus was densely innervated (Fig. 12A, C–E). The nerve endings appeared to be fine in all arborization areas. The columnar connections of CL2 neurons between the PB and the CBL corresponded to the topographical scheme of Fig. 10D for the CL1 neurons, but no CL2 neurons that crossed the midline in the y- or z-bundles between the PB and CBL were stained.

Discussion

The central complex (CX) is one of the most regularly organized neuropils in the insect brain and, as increasing evidence suggests, appears to play a role in visual integration and motor control (Homberg 1985, 1994a; Milde 1988; Strauss et al. 1992; Strauss and Heisenberg 1993; Bausenwein et al. 1994). In contrast, the neuronal architecture of the CX and its connections to other brain areas are only poorly understood. This study builds upon earlier work by Williams (1972, 1975) on the organization of the CX in the locust and provides a survey of neuronal cell types innervating a subcompartment of the CX, viz., the CBL. We show that the CBL of the locust is composed of six layers that are connected through five types of tangential neurons with specific areas in the LALs. In addition, two systems of columnar neurons provide connections with the PB, parts of the noduli, and the lateral triangle of the LALs.

We have chosen two different techniques, Golgi impregnation and single-cell injection with Lucifer yellow, to stain neurons of the CBL. The Golgi technique leads to stochastic impregnation of single cells, but some neuron types may be stained more often than others, and especially large neurons are often impregnated only partly (Strausfeld 1980). In our Golgi preparations, for example, TL1 cells have been stained only once, and TL5 neurons are not stained at all. Lucifer-yellow injections usually lead to complete staining of single cells. With this technique, however, the penetration of neurons with large axons is more likely than dye injection of small fibers, e.g., the small TL4 neurons, are not stained following Lucifer injections. The combination of both staining procedures may, thus, have maximized the yield of neuronal cell types of the CBL, but other types may also exist. Additional large-field neurons with diffuse arborizations in the CBL, such as the neuron in Fig. 9, may be present. The projection patterns of tangential and columnar neurons of the CBL reveal organizational properties of the CBL that might have important functional implications.

Tangential neurons

The tangential neurons are mainly responsible for the layering of the CBL. The six layers seen following Bodian staining correspond to the six layers revealed by the branching patterns of the TL neurons. TL neurons with arborizations restricted to individual layers are found for layers 1, 2, and 5, but not for layers 3, 4, and 6. The boundary between layers 3 and 4 appears to be formed by TL neurons whose arborizations extend through layers 3 and 4 or through layers 4 and 5. Correspondingly, the border between layers 3 and 4 is not as clear following Bodian staining as are the limits of the other layers. Apart from TL1 and TL5 neurons, layer 6 is exclusively innervated by TL3 neurons, which also arborize in layer 2.

All TL neurons have additional ramifications in the ipsilateral LAL of the brain. Whereas this is the only arborization area outside the CBL for neurons TL1–TL4, TL5 neurons also innervate the PB and the superior protocerebrum. The LAL of *S. gregaria* is compartmentalized into an anterior and a posterior dorsal shell, a ventral shell, the median olive, and the lateral triangle (Homberg 1991, 1994a). TL neurons connect the CBL only to the anterior dorsal shell (TL4 and TL5 neurons), the median olive (TL3 neurons), and the lateral triangle (TL1, TL2, and presumably TL5 neurons).

Whereas TL1 and TL5 neurons provide global connections to most or all CBL layers, TL2–TL4 neurons maintain, in part, layer-specific connections; this suggests that functional differences exist between the CBL layers. Layer 1 of the CBL is specifically connected by

TL4 neurons to the dorsal shell of the LAL. Vitzthum et al. (1996) have recently shown in *S. gregaria* that neurons that closely resemble TL4 neurons exhibit immunostaining with an antiserum against the peptide allatostatin I of *Diploptera punctata*. Through the various subtypes of TL2 neurons, layers 2–5 of the CBL are connected to small areas in the lateral triangle of the LAL. TL2 neurons with arborizations in layers 3 and 4 innervate more distal areas of the lateral triangle, whereas TL2 cells with ramifications in layer 2 innervate more medial areas of the lateral triangle. These two findings indicate that the lateral triangle is further subdivided and that topographically ordered connections between subfields of the lateral triangle and specific layers of the CBL might exist. Layer 2 and layers 4–6 of the CBL are, in addition, connected through TL3 neurons to the median olive. In view of the small number of TL3 neurons found for each layer, TL3 neurons projecting to layer 3 may also exist. A large number of tangential neurons in the CBL of the locust exhibits immunoreactivity against gamma aminobutyric acid (Homberg 1994b). These neurons apparently correspond to TL2 and TL3 neurons. TL1 neurons arborize throughout layers 2–6 or 2–5 of the CBL and throughout the lateral triangle of the LAL and, therefore, have a clearly different morphology from that of TL2 neurons, which connect single layers of the CBL to small areas of the lateral triangle. TL5 neurons can be distinguished from the other TL neurons by several features. They innervate all layers of the CBL, have cell bodies in the pars intercerebralis, and have more than one arborization field outside the CBL. Two pairs of CBL neurons with a morphology similar to that of TL5 neurons are dopamine-immunoreactive (Wendt and Homberg 1992).

The nerve endings of all TL neurons in the CBL are beaded. Electron-microscopic investigations have revealed that structures visible in the light microscope as swollen, varicose, or clavate terminals are regions of mainly synaptic output, whereas fine or spiny terminals predominantly receive synaptic input (reviewed in Strausfeld 1976). The beaded endings of all TL neurons in the CBL therefore suggest the presence of regions of synaptic output. In contrast, the fine ramifications in the dorsal shell of the LAL in TL4 and TL5 neurons, in the lateral triangle of TL1 neurons, and the dense tufts of very fine branches of TL2 and TL3 neurons in the lateral triangle and the median olive indicate regions of dendritic input. Therefore, TL neurons might provide synaptic input into the CBL.

Neurons that appear to be homologous with the TL neurons have been described in the CX of *D. melanogaster* (Hanesch et al. 1989). In the doughnut-shaped ellipsoid body, which corresponds to the CBL of the locust, Hanesch et al. (1989) have found several types of ring neurons that resemble the TL2 and TL3 neurons of the locust. They arborize with bleb-like endings throughout various ring-like zones of the ellipsoid body. Outside the ellipsoid body, ring neurons ramify in a small neuropil area, viz., the “lateral triangle”, which corresponds in position to the median olive of the locust. Within this area, the ring neurons have a “very small, exceptional compact bush of thin fibers” (Hanesch et al. 1989), just as we have found for TL2 and TL3 neurons in the median olive and lateral triangle.

Columnar neurons

Two types of columnar neurons are found in the CBL of *S. gregaria*. Both connect three neuropils: the PB, the CBL, and the lateral triangle of the LAL (CL1 neurons) or the lower subunit of the nodulus (CL2 neurons). The CL1 neurons correspond to the system of 64 CC1 neurons described by Williams (1975). On the basis of ethyl-gallate staining, Williams (1975) has shown that the PB and CBL are divided into 16 columns, that the PB columns are connected in a topographical order by CC1 neurons to columns in the CBL, and that axonal fibers from the CC1 neurons continue to the contralateral LAL. Williams (1975) has further shown that each column of the PB is innervated by four neurons with an identical fiber course. Our Golgi impregnation of and Lucifer-yellow injections into single members of this system provide further information on the morphology of the CC1/CL1 neurons. The columnar arborizations of these neurons are not strictly delineated from each other in either the PB or the CBL. In the CBL, the lateral extensions into neighboring columns are more pronounced in layers 1, 2, and 6 than in layers 3, 4, and 5. This shows that CL1 neurons contribute to the layering of the CBL, as do TL neurons, and again points to possible functional differences between the CBL layers. The nerve endings in the CBL usually have a fine appearance following both Golgi impregnation and Lucifer-yellow staining, suggesting that CL1 cells receive neuronal input in the CBL. In the LAL, CL1 neurons terminate in small areas of the lateral triangle. This provides further evidence for a topographical organization of the lateral triangle, but we have not been able to determine whether the subfields innervated in the lateral triangle are spatially related to specific columns in the PB or CBL. The nerve endings in the lateral triangle are bleb-like, suggestive of regions of synaptic output.

The second type of columnar neurons of the CBL in *S. gregaria*, the CL2 neurons, can be regarded as intrinsic to the CX, because they connect the PB and CBL to the noduli. CL2 neurons have only been found in nine preparations. In all examples, columns in the PB are connected to columns in the CBL with an arrangement similar to that of the CL1 neurons. All neurons project to the contralateral nodulus. Although CL2 neurons crossing the midline between the PB and CBL have not been stained in our preparations, they might form a system comparable with that of the CL1 cells. In an analysis of one of the two fascicles of the w-bundle, Williams (1972) has shown four fibers with projections to the CBL and the lower subunit of the nodulus, suggesting that the CL2 fiber system consists of four neurons per column or 64 neurons in total, like the CL1 fiber system. In addition to the CL1 and CL2 fibers, Williams (1972) has reported four fibers projecting to the CBL in the w-bundle. These CC1 accessory fibers apparently terminate in the CBL, but they have not been encountered in our study.

In *D. melanogaster*, Hanesch et al. (1989) have listed 8–10 types of columnar neurons of the ellipsoid body. Two types exhibit similarities to the CL1 neurons of the locust. One of these (the eb-pb-vbo neurons) has arborizations with beaded endings in the PB and in the ventral body and ramifications with spiny terminals in the ellipsoid body. The second type (the pb-eb-ltr neurons) has arborizations in the PB and ellipsoid body and projects to the contralateral lateral triangle. Columnar neurons corresponding to CL2 neurons have also been observed in *D. melanogaster*, but in contrast to the locust neurons, these neurons have arborizations in the ipsilateral nodulus (Hanesch et al. 1989). Other types of columnar neurons in *D. melanogaster* have arborizations in the ellipsoid body and in the fan-shaped body, which corresponds to the CBU of the locust (Hanesch et al. 1989). Similar neurons have not yet been found in the locust (Williams 1972, 1975; this study).

Functional implications

Systems of neurons linking columns of the PB to columns of the CBL or CBU in a topographical order are a conspicuous feature of the CX in the locust and other insects (Strausfeld 1976; Mobbs 1985; Hanesch et al. 1989; Homberg 1991; Schildberger and Agricola 1992; Vitzthum et al. 1996). In *S. gregaria*, the arborizations of CL1 neurons innervating the right and left PB hemispheres alternate and overlap in the CBL. Similar innervation patterns have been found for columnar cell systems in the CBU of *S. gregaria* (Homberg 1991; Vitzthum et al. 1996). The functional significance of these innervation patterns is not understood, but they allow specific exchange and processing of information from the right and left brain hemispheres. Experimental evidence suggests a role of the central complex in motor control and visually guided behavior (Huber 1960a, b; Otto 1971; Strauss et al. 1992; Strauss and Heisenberg 1993; Bausenwein et al. 1994; Homberg 1994a; Ilius et al. 1994). Both tasks may involve the precise integration of information from the two brain hemispheres.

The common projections of CL1, TL1, and TL2 neurons to the lateral triangle of the LAL opens the possibility for feedback loops between columnar and tangential neurons of the CBL. This is supported by the observation that TL2 neurons show beaded endings in the CBL and tangles of fine processes in the lateral triangle, whereas CL1 neurons exhibit beaded endings in the lateral triangle and fine processes in the CBL.

Another conspicuous results of this study is the apparent lack of direct connections between the CBL and CBU. Both subunits of the CB appear to receive common parallel input from the PB through columnar neurons (see also Williams 1972; Homberg 1991; Vitzthum et al. 1996). On the other hand, connections of the CBL and CBU to the noduli and to the LAL are topographically largely distinct. The CBL is connected to the dorsal shell, the median olive, and the lateral triangle of the LAL, and to the lower subunits of the noduli. In contrast, the CBU is mainly connected to the dorsal and ventral shells of the LAL, and to the upper subunits of the noduli (Homberg 1991; Wendt and Homberg 1992; Dircksen and Homberg 1995; Vitzthum et al. 1996). Other than the PB, only the dorsal shell of the LAL might therefore be innervated both by neurons of the CBL and by neurons of the CBU. A few tangential neurons appear to arborize in the CBU and in the CBL (Fig. 9), but these neurons seem to provide a common input rather than a direct connection between both neuropils. The lack of direct projections between the CBL and CBU, and the spatially distinct connections of the CBL and CBU to the noduli and to the LALs, therefore, indicate parallel rather than serial processing of neuronal information in the CBL and CBU of *S. gregaria*. In contrast, Hanesch et al. (1989) have reported a variety of columnar cells connecting the ellipsoid body and the fan-shaped body of *D. melanogaster*, and several neurons arborizing in both the ellipsoid body and the fan-shaped body have also been described in the fly *Musca domestica* (Strausfeld 1976).

Anatomical investigations in several insect species have revealed direct and indirect connections from the optic lobes to the CX (Honegger and Schürmann 1975; Strausfeld 1976; Williams 1972; Homberg et al. 1990, 1991). Neurons that exhibit serotonin-like, pigment-dispersing hormone-like, and allatostatin-like immunoreactivity link the posterior optic tubercles to the PB in *S. gregaria* (Homberg 1991; Homberg et al. 1991; Vitzthum et al. 1996). In *Manduca sexta*, the anterior optic tubercles are connected to the LALs through FMRF-amide-immunoreactive neurons (Homberg et al. 1990). Recently, we have shown that tangential and columnar neurons of the CBL of *S. gregaria* are sensitive to polarized light and show sinusoidal e-vector response functions, with those e-vectors eliciting maximal and minimal spike activity being 90° apart (Müller and Homberg 1994; Homberg and Müller 1995). Ants and bees, and probably other insects, use the polarization pattern of the blue sky, such as the position of the sun, for compass navigation and path integration (reviewed by Rossel 1993; Wehner 1994). The neuronal mechanisms underlying polarized-light navigation are poorly understood, and the brain areas involved in these calculations are still unknown. Interestingly, recent computational models of neuronal networks capable of path integration with the aid of a sky compass have generated neural architectures that share several features with the CX, including rows of repetitive neuronal elements with specific columnar and global connections, and recurrent feedbacks (Wittmann and Schwegler 1995; Hartmann and Wehner 1995). In the locust, Eggers and Weber (1993) have demonstrated menotactic e-vector orientation in walking activity, but detailed behavioral and physiological data on polarized-light navigation in the locust are still lacking. Future behavioral and physiological experiments should show whether and how the CX of the locust is involved in e-vector navigation and path integration. Anatomical investigations, such as ours, may help to clarify the functional capacities of the CX and should facilitate future physiological analyses of this prominent structure in the insect brain.

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