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# Central Projections of First-Order Ocellar Interneurons in the Bug *Triatoma infestans* (Heteroptera: Reduviidae)

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*ABSTRACT* The projections of first-order ocellar interneurons were analyzed in the hematophagous bug *Triatoma infestans* by cobalt filling. The axons run between the calyces of the mushroom bodies and dorsal of the central body to different regions of the brain and the subesophageal and thoracic ganglia. The interneurons can be grouped into large L cells and small S cells. The L cells have cell bodies ranging from 11.5 to 25 μm and axons ranging from 8 to 25 μm diameter (measured in the ocellar nerve); the S cells have smaller cell bodies of 9 μm or less and axon diameters less than 5 μm. The projections of ten L cells are described in detail; they project to the protocerebral posterior slope (PS), the other ocellus (O), the optic neuropile, and the subesophageal, pro-, meso-, and metathoracic ganglia, either to ipsi- (PS I, II), or contra- (PS IV, V), or bilateral areas. In this case projections occur to the same areas (PSO, PS III) or different areas at each side (PSOE; E = eye). Large-descending (LD) first-order interneurons project to the contralateral posterior slope of the protocerebrum, the deutocerebrum, and subesophageal, pro-, meso-, and metathoracic areas (LD I–III). Cell bodies are located in the dorsal protocerebral lobes and pars intercerebralis, except the PS II neuron and three LD cells, which are located in the ipsilateral posterior protocerebrum. This is the first report about ocellar pathways in Hemiptera. Their adaptive function is discussed with reference to the bugs' behavior as Chagas disease vectors. 

Insect ocelli are intriguing photoreceptors; their function, however, is still enigmatic despite numerous anatomical, physiological, and behavioral investigations (reviewed by Goodman, '70, '81; Mizunami, '94). Different functions have been ascribed to ocelli, in particular in the "good flyers," e.g., they modulate the tonus of the flight system, control flight equilibrium (Wilson, '78; Stange and Howard, '79; Stange, '81; Rowell and Pearson, '83), and are possibly involved in the dorsal light response used during flight (Kalmus, '45; Goodman, '65). They are also involved in the orientation of walking flies (Wehrhahn, '84) and ants (Fent and Wehner, '85).

One approach to study the function of a sense organ is to look at its projections in the central nervous system. The interneurons of the ocellar system have been studied in Blattoidea, Orthoptera, Lepidoptera, Odonata, Hymenoptera, Diptera, and Trichoptera (for references, see Goodman, '81; Koontz and Edwards, '84; Nässel and Hagberg, '85; Hagberg and Nässel, '86). Nevertheless, no data on the ocellar pathways of Hemiptera are at present available.

Several reasons make the hematophagous bug *Triatoma infestans* an interesting model for a comparative analysis of the ocellar system: 1) these bugs are usually not considered among the "good flyers" insects, as judged by their habits, 2) they have two well-developed ocelli behind their compound eyes (most insects analyzed up to now have three ocelli), 3) in contrast to entirely diurnal or nocturnal insects, *T. infestans* exhibits a rhythm of activity that is split into a dusk and a dawn component (Lazzari, '92), being exposed to wide changes of light intensities, and 4) only a few data are available on the ocelli of Hemiptera (Goodman, '81).

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In this study the projections of first-order ocellar interneurons within the brain and thoracic ganglia of the bug *T. infestans* are described based on cobalt fillings.

## MATERIALS AND METHODS

The experimental animals were males of *T. infestans* reared in the laboratory at 30°C and 50–70% relative humidity, and fed on citrated sheep blood. A total of 120 bugs were used for $CoCl_2$ filling of ocellar interneurons. The insects were immobilized with plasticene on a glass plate, dorsal side up, and the head was fixed with dental wax. The ocellar lens was cut off with a razor blade and the underlying neuropile was pierced with a thin needle. A small drop of 2.5% cobalt chloride in distilled water was placed on the lesion, which was then covered with Vaseline. Cobalt applications were done to one ocellus or to both ocelli. The preparations were left for 1–3 h at 21°C for cobalt uptake. Thereafter, the insect was fastened by its back on a wooden plate with dental wax, ventral side up. The brain and ganglia were partially exposed and immersed for 5 min in a solution of ammonium sulfide (5 drops of saturated ammonium sulfide added to 5 ml Ringer). The dissected nervous system was fixed for 2 h in glacial acetic acid/ethanol/formalin fixative (Lillie, '65) and silver intensified according to Bacon and Altman ('77). The cobalt-labeled tissue was either cleared with methyl salicylate for whole mount viewing, or embedded in Durcupan and cut horizontally at 30–35 μm. Photographs and reconstruction drawings were made from whole mounts and sections.

The neurons were named in accordance to the areas of the central nervous system to which they project, i.e., PS for posterior slope of the protocerebrum, LD for large-descending, E for eye, O for ocellus. Nervous areas were named according to Strausfeld ('76).

## RESULTS

The two ocelli of *T. infestans* are located in an unusual position, i.e., behind the compound eyes, looking dorsolaterally and frontally. The ocelli are well developed with prominent asymmetric ovoid lenses (diameters 450 × 280 μm) overlying the photoreceptor layer. The entire lateral membrane of each retinula cell is modified to form microvilli at its distal half. The microvilli of adjacent cells are connected, giving place to a hexagonal meshwork of rhabdomeres across the photoreceptor cell layer. The axons of the photoreceptors synapse with second-order neurons in the peripheral neuropile at the base of the ocelli. Each neuropile is connected to the brain by an ocellar nerve 440 μm long and 65 × 43 μm thick (Insausti, '93, '94). The fibers of the ocellar nerves continue inside the brain as lateral tracts, through the superficial dorsal protocerebrum. They run between the calyces of the mushroom bodies and dorsal of the central body to different regions of the brain, and the subesophageal and thoracic ganglia. First-order interneurons can be classified into large L cells and small S cells. The L cells have cell bodies ranging from 11.5 to 25 μm and axons ranging from 8 to 25 μm in diameter (measured in the ocellar nerve). The S cells are smaller; their cell bodies are 9 μm or less in diameter and their axons less than 5 μm.

### *Central projections of ocellar interneurons*

The projections of ten different types of L cells were analyzed in detail and described as five PS neurons (PS I–V), which project to, and arborize in, the ipsi- and contralateral posterior slope neuropiles of the protocerebrum (Fig. 1A,B); one PSO neuron, which projects to and arborizes in the ipsi- and contralateral posterior slope neuropiles of the protocerebrum and to the contralateral ocellus (Fig. 2A); one PSOE neuron which projects to, and arborizes in, the ipsilateral posterior slope neuropile of the protocerebrum, the contralateral ocellus, and the optic lobes (Fig. 2B); and three LD neurons (LD I–III), which descend to the thoracic ganglia and arborize in the posterior slope of the protocerebrum and deutocerebrum, and the subesophageal and thoracic ganglia (Fig. 3).

### *PS I neuron*

The axon of the PS I neuron has a diameter of 2.6 μm; it arborizes in an area of the ipsilateral posterior slope of the protocerebrum that lies more medially compared to the PS II neuron (see below). The cell body has a diameter of 16 μm and is located in the ipsilateral posterior slope of the protocerebrum, dorsal to the endings of the fibers (Figs. 1A, 4A,B).

### *PS II neuron*

The axon of the PS II neuron has a diameter of 2.6 μm; it runs to the ipsilateral

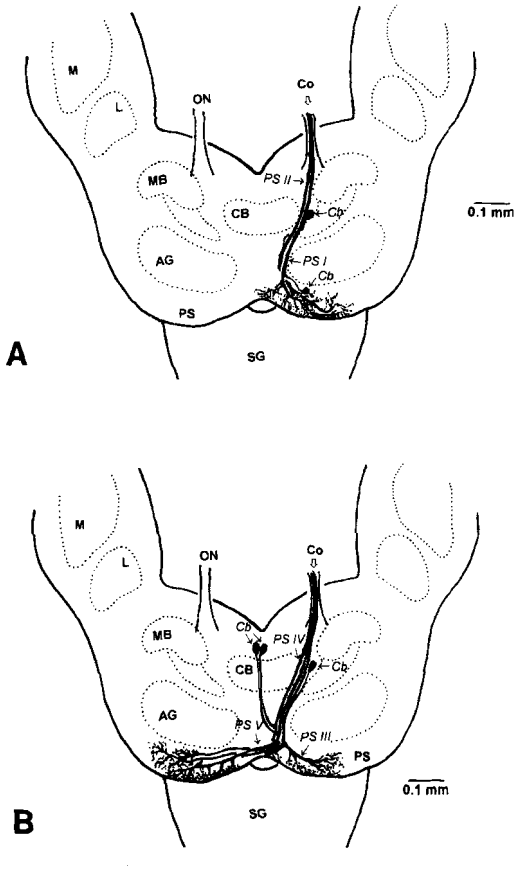


Fig. 1. Diagram of the brain of *Triatoma infestans* (dorsal view) showing cobalt-filled ocellar PS interneurons. **A:** Projections of PS I and PS II neurons. They arborize in the ipsilateral posterior slope of the protocerebrum. Their cell bodies are located in the ipsilateral posterior slope (PS I) and at the lateral side of the ocellar tract (PS II). **B:** Projections of PS III–V interneurons. The cell body of the PS III neuron is located at the lateral side of the ocellar tract, and the cell bodies of the PS IV and PS V neurons in the pars intercerebralis near the midline of the brain. AG, antennal glomerulus; Cb, cell body; CB, central body; Co, place of cobalt application; L, lobula; M, medulla; MB, mushroom body; ON, ocellar nerve; PS, posterior slope of the protocerebrum; SG, subesophageal ganglion.

Fig. 2. Diagram of the brain of *Triatoma infestans* (dorsal view) showing cobalt-filled ocellar PSO and PSOE interneurons. Their cell bodies lie in the pars intercerebralis. **A:** Projection of a PSO neuron with arborizations in the ipsi- and contralateral posterior slope of the protocerebrum. **B:** PSOE neuron with branches to the ipsi- and contralateral ocellar tract, the ipsilateral posterior slope of the protocerebrum, and the contralateral lobula neuropile. AG, antennal glomerulus; Cb, cell body; CB, central body; Co, place of cobalt application; L, lobula; M, medulla; MB, mushroom body; ON, ocellar nerve; PS, posterior slope of the protocerebrum; SG, subesophageal ganglion.

posterior slope of the protocerebrum. From this fiber, arborizations extend laterally into the posterior slope. The cell body is 22 μm in diameter and is situated dorsally in the protocerebrum at the lateral side of the ocellar tract (Figs. 1A, 4A,B).

### *PS III neuron*

The axon of the PS III neuron has a diameter of 2.5 μm and bifurcates at the ipsilateral posterior slope of the protocerebrum and finally arborizes there ipsi- and contralaterally. The crossing takes place dorsally to the esophageal foramen, above the commissure. In the ipsilateral posterior slope the arborization pattern is similar to that of the PS I neuron. The cell body has a diameter of 22 μm and, as with the PS I cell body, lies dorsally in the protocerebrum, lateral to the ocellar tract (Figs. 1B, 4A,B).

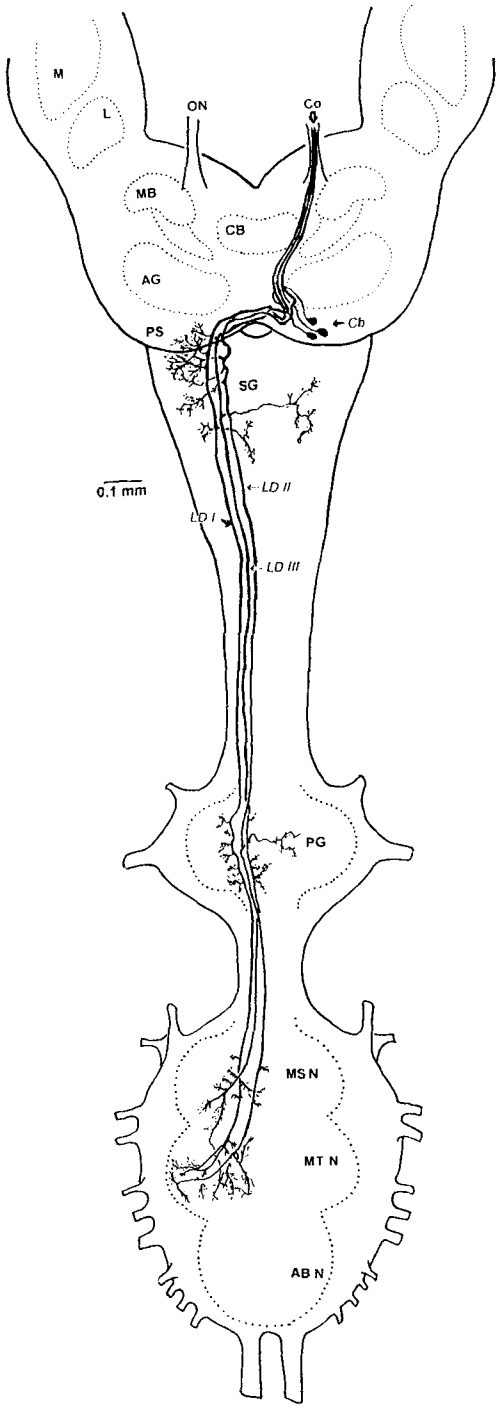


Figure 3

### *PS IV and PS V neurons*

The axons of PS IV and PS V neurons have a diameter of 2.7 μm and run to the ipsilateral posterior slope of the protocerebrum. From there, they continue to, and arborize in, the posterior slope of the contralateral protocerebrum, in a similar pattern as the PS III neuron. The fiber of the PS IV neuron arborizes in the dorsal area of the posterior slope, and that of the PS V neuron more ventrally. Their cell bodies have a diameter of 25 μm and are located in the anterior part of the pars intercerebralis, near the midline of the brain, above the central body (Figs. 1B, 4A,B).

### *PSO neuron*

The PSO neuron has four branches which diverge in an X-shape fashion in the midline of the brain to run in four directions: to the ipsi- and contralateral ocellar tract and the ipsi- and contralateral posterior slope of the protocerebrum. The branches are 7 μm thick. The cell body has a diameter of 11.5 μm and is located in the anterior part of the pars intercerebralis, near the midline of the brain, above the central body (Figs. 2A, 4A,B).

### *PSOE neuron*

The projection pattern of the PSOE neuron is similar to that of the PSO neuron. However, the posterior contralateral branch runs up to the dorsal neuropile of the optic lobula (instead of ending in the posterior slope of the protocerebrum); it is much thinner (1.6 μm) than the ipsilateral branch (3.2 μm). The cell body of the PSOE neuron has a diameter of 13.8 μm and is located in the pars intercerebralis, near the midline of the brain (Figs. 2B, 4A–C).

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Fig. 3. Diagram of the brain and thoracic ganglia of *Triatoma infestans* (dorsal view) showing three cobalt-filled LD interneurons. Their cell bodies (Cb) lie in the ipsilateral posterior slope of the protocerebrum. The axon fibers project to the contralateral posterior slope of the protocerebrum and deutocerebrum. The axons descend contralaterally, sending branches to the subesophageal (SG), pro-, meso-, and metathoracic ganglia (PG, prothoracic ganglion; MS N, mesothoracic neuropile; MT N, metathoracic neuropile; AB N, abdominal neuropile); there, they arborize within dorsal neuropiles. AG, antennal glomerulus; CB, central body; Co, place of cobalt application; L, lobula; M, medulla; MB, mushroom body; ON, ocellar nerve; PS, posterior slope of the protocerebrum.

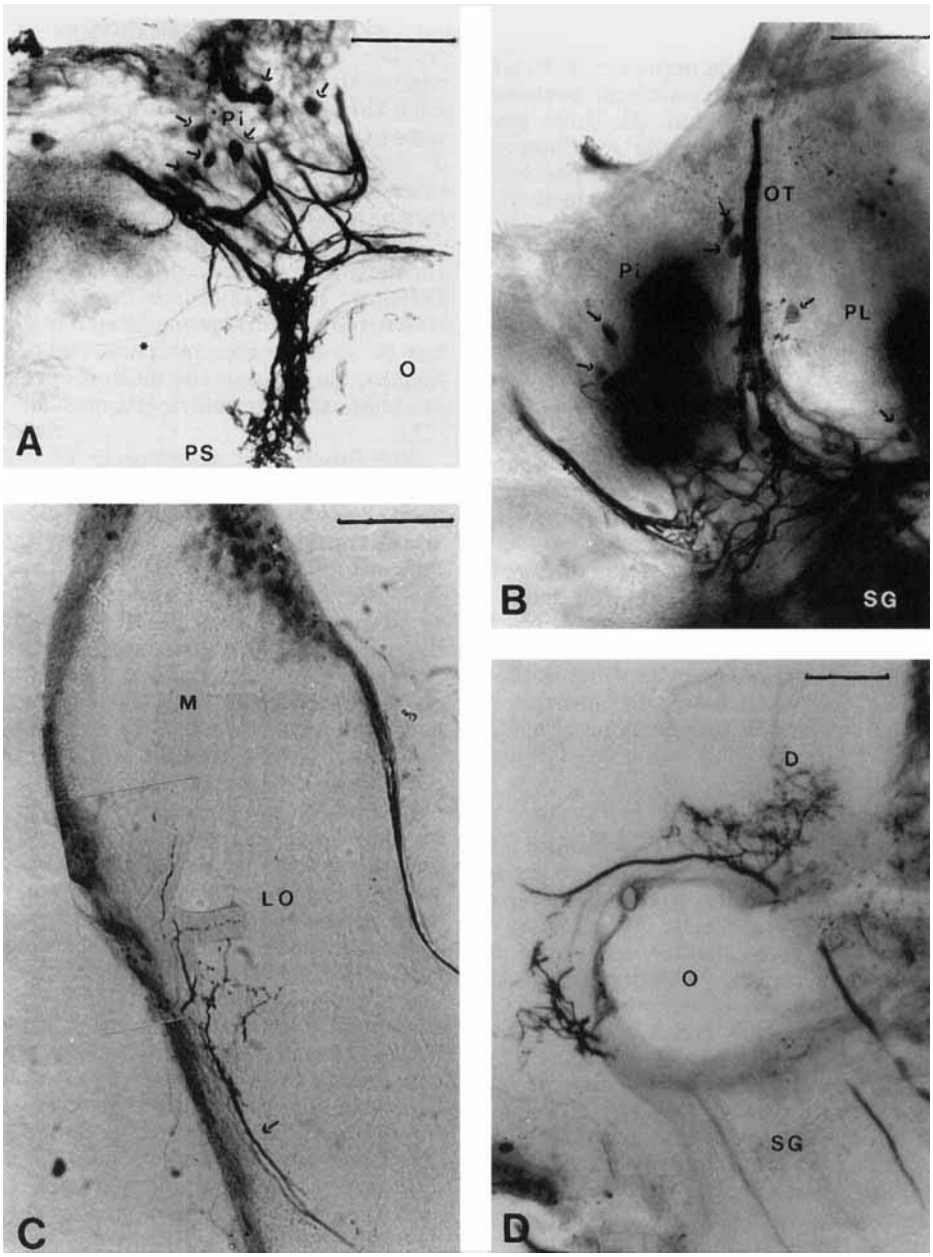


Fig. 4. *Triatoma infestans.* Micrographs of cobalt-filled interneurons from injections of one lateral ocellus (**A–C**) or both ocelli (**D**). **A,B:** Whole mount preparations showing cell bodies (arrows) and neurites of PS I–V, PSO, and PSOE neurons (dorsal view). Note rich arborizations in the posterior slope of the protocerebrum and the projection from the PSOE neuron to the optic lobula (asterisk). **C,D:** 25–30 μm horizontal sections. C: Details of termination of a PSOE fiber (arrow) in the optic lobula. D: Details of arborization of an LD II fiber in the deutocerebrum. D, deutocerebrum; LO, lobula; M, medulla; O, esophagus; OT, ocellar tract; Pi, pars intercerebralis; PL, protocerebral lobes; PS, posterior slope of the protocerebrum; SG, subesophageal ganglion. Scale bars = 100 μm (A–C); 50 μm (D).

*LD I–III neurons*

The cell bodies have a diameter of 16 μm and are located in the ipsilateral posterior slope of the protocerebrum. All three neurons have extensive, partially overlapping, contralateral arborizations in the posterior slope of the protocerebrum and in the deutocerebrum. The axons have diameters of 4.6, 4.6, and 6.9 μm; they cross through the commissure dorsal to the esophageal foramen, to project via the contralateral esophageal connective to the subesophageal ganglion. There they give off stout branches, ipsi- (LD III) and contralaterally (LD I and LD II), and continue via the cervical connective to the thoracic ganglia where they arborize within the dorsal neuropiles of the pro-, meso-, and metathoracic ganglia. At the prothoracic neuropile, each cell arborizes in a characteristic fashion. The LD I neuron sends multiple branches, while the LD II a single one, both contralaterally. The LD III neuron sends a single branch to the ipsilateral prothoracic neuropile. Despite running in an almost parallel fashion, the three neurons differ in their projections into each neuropile and can be individualized in the preparations (Figs. 3, 4D, 5A–E, 6A,B).

## DISCUSSION

In the ocellar system of *T. infestans* two kinds of first-order interneurons are recognized, grouped according to the diameter of the fibers, i.e., L and S cells. The L fibers of this species are more slender than those observed in other insects, reaching up to 7 μm diameter in the central nervous system (in the ocellar nerve, they reach up to 25 μm). They project to ipsi- (PS I, II), contra- (PS IV, V), and bilateral areas. In this case projections occur to the same bilateral centers (PSO, PS III) or different zones at each side (PSOE). Descending first-order interneurons project to the contralateral posterior slope of the protocerebrum, deutocerebrum, and subesophageal, pro-, meso-, and metathoracic centers (LD I–III).

Cell bodies locate in the dorsal protocerebral lobes and pars intercerebralis, except PS II neurons and the three LD cells, which locate in the ipsilateral posterior protocerebrum.

Compared with insects with three ocelli, the ocellar system of *T. infestans* reveals two interesting differences: 1) whereas in *T. infestans* direct reciprocal projections occur between both lateral ocelli, these projections are absent in insects with three ocelli, where the lateral ocelli are connected by interneurons of the median ocellus; and 2) in insects with three ocelli, bilateral projections originate exclusively from the median ocellus. In *T. infestans,* in contrast, both ocelli have bilateral projections, into paired bilateral centers or into different centers at each side.

The cockroach *Periplaneta americana* and the moth *Trichoplusia ni* also have two ocelli. Different from *Triatoma infestans,* in the cockroach projections are formed not by first- but by second-order interneurons (Toh and Sagara, '84), and in the moth all projections are bilaterally symmetric (Pappas and Eaton, '77).

The function of insect ocelli has been related to flying. Indeed, in insects signed as good flyers, ocellar inputs are involved in fly stabilization (Wilson, '78; Stange and Howard, '79; Stange, '81; Rowell and Pearson, '83). Although the ability of *T. infestans* to sustain quite long dispersive flights has been described (Lehane and Schofield, '78; Schofield et al., '92), the aerial medium is not the most important in the life of this species, as it is in other insects such as flies or bees. Triatominae are mainly walking insects, which ensue dispersing flights under certain conditions (e.g., starvation), and can hardly be ascribed as belonging to the "good flyers" group. However, such striking differences in habits from those insects do not correspond with differences in the ocellar pathways.

Insects in which ocellar systems were investigated exploit all the temporal niches; some are diurnal (crickets, flies, bees, wasps) or nocturnal (cockroaches, moths), others are active at dusk and dawn (trichopterous). In the cabbage moth *Trichoplusia ni,* input from the ocelli determines the threshold light intensity for flight and makes adjustments to small light-phase changes (Eaton et al., '83). *Triatoma infestans* exhibits spontaneous locomotory activity twice a day, at dusk and dawn (Lazzari, '92). Concerning flight, laboratory experiments under discrete light/dark cycles showed an initial activity immediately after light-off (Lehane and Schofield, '82), but the relationship between the ocellar system and flight remains to be investigated since the analysis of the ocellar projections does not reveal correlation between the complexity of the ocellar system and the daily activities of this insect.

Small differences in the ocellar system have been reported for the hemimetabolous Blat-

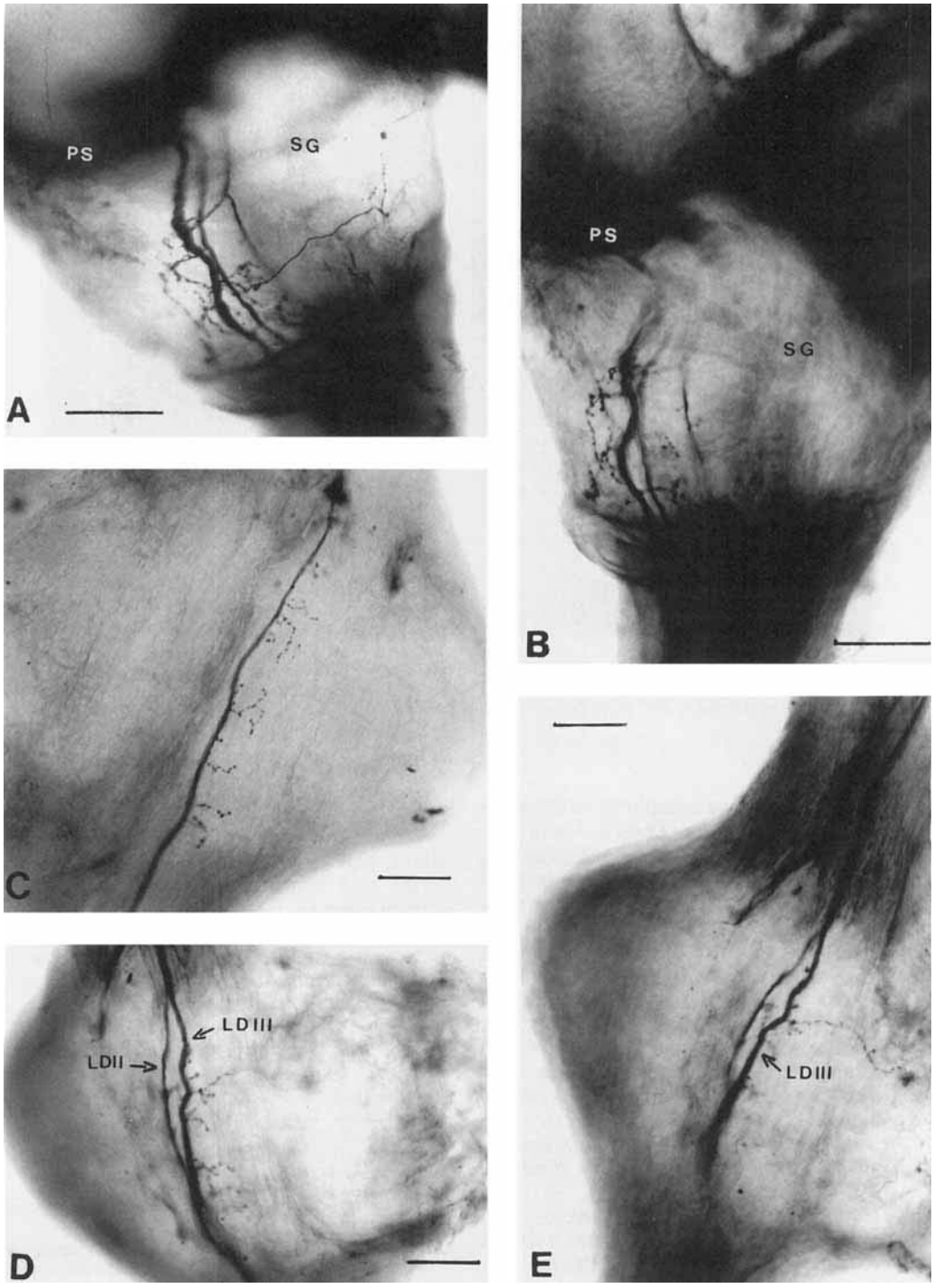


Fig. 5. *Triatoma infestans.* Micrographs of cobalt-filled LD interneurons from unilateral injections (whole mount preparations, dorsal view). **A,B:** Details of arborization of LD fibers in the subesophageal ganglion. **C–E:** Arborization of LD fibers in the prothoracic ganglion. PS, posterior slope of the protocerebrum; SG, subesophageal ganglion. Scale bars = 50 μm (A,B); 25 μm (C–E).

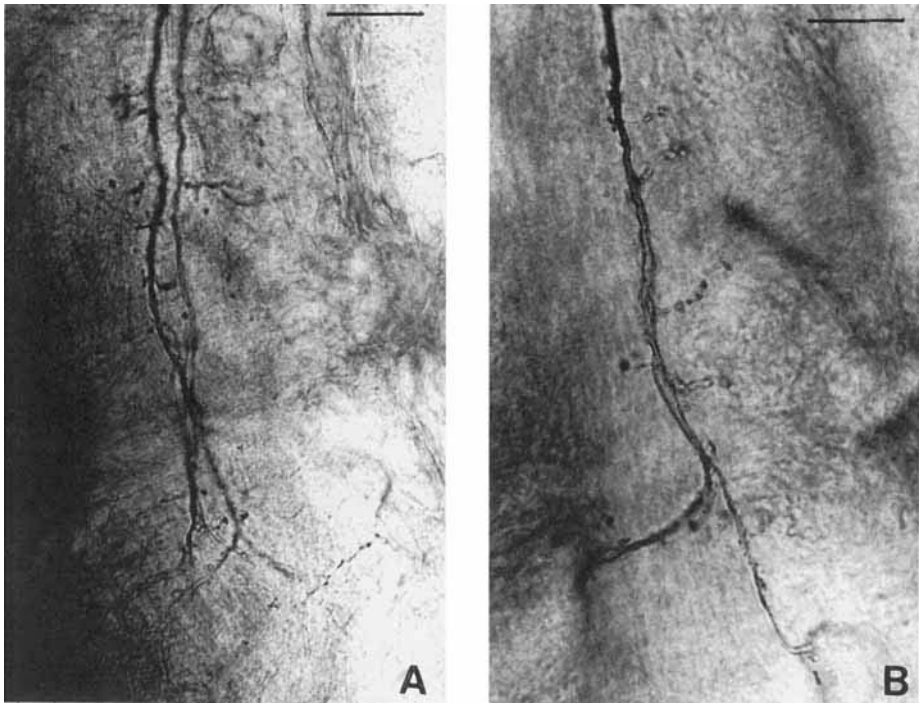


Fig. 6. *Triatoma infestans.* Micrographs of cobalt-filled LD interneurons from unilateral injections (whole mount preparations, dorsal view). Details of arborizations of fibers from LD neurons in the meso- and metathoracic neuropiles. **A:** LD I and LD II. **B:** LD III. Scale bars = 25 μm.

toidea and Orthoptera compared to Odonata and the holometabolous Diptera, Hymenoptera, Lepidoptera, and Trichoptera (Hagberg and Nässel, '86). The differences are evident in the development of the ocelli, the contralateral projections, and the division of the median ocellus. For the Hemiptera, this report on *T. infestans* is the first available. This species has well-developed ocelli, complex first-order projections, including contralateral and descending pathways, but relatively small diameter fibers; thus, it is quite complex, but slower than other ocellar systems with thicker fibers. Fibers reach up to 7 μm in the central nervous system of *T. infestans,* whereas in Trichoptera, e.g., they measure up to 10 μm (Hagberg and Nässel, '86). The descending projections to pro-, meso-, and metathoracic motor centers suggest a direct relationship with locomotion or flight. Further work on flight control should reveal whether the ocelli of *T. infestans* play a role similar to that proposed for other insect species.

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