The Terminal Abdominal Ganglion of the Wood Cricket *Nemobius sylvestris*

Teresita C. Insausti,* Claudio R. Lazzari, and Jérôme Casas

Institut de Recherche sur la Biologie de l'Insecte, UMR 6035 CNRS – Université François Rabelais, Tours, France

ABSTRACT The abdominal cerci of the wood cricket, *Nemobius sylvestris*, are covered by a variety of hair-like sensilla that differ in length, thickness, and articulation. Fillings from the cercal nerves with cobalt chloride and fluorescent dyes revealed the projection of sensory axons into the terminal abdominal ganglion of the ventral nerve chain. Two projection areas on each side of the terminal abdominal ganglion midline could be identified: a posterior cercal glomerulus and an anterior bristle neuropil. Axons from some cercal sensilla ascend through the connectives to reach the metathoracic ganglionic mass. As their axons pass through each segmental abdominal ganglion, they project medial arborization. Cross-sections of the terminal abdominal ganglion and retrograde fills with cobalt chloride and fluorescent dyes from connectives revealed several small cells and seven pairs of giant ascending interneurons organized symmetrically. Giant somata are located contralateral to their axons (diameters between 20 and 45 μm). The cercal projections overlap extensively with the dendritic fields of the giant interneurons. In the terminal abdominal ganglion, we identified nine longitudinal tracts, two major tracts, and seven smaller ones. The functional implications of the neuranatomical organization of the system are discussed on a comparative basis. J. Morphol. 000:000–000, 2008.

KEY WORDS: cricket; neuroanatomy; giant interneurons; mechanoreceptor projections; terminal abdominal ganglion

Insects such as cockroaches, locusts, crickets, and others possess a pair of cerci bearing air-sensitive filiform sensilla at the end of the abdomen. When stimulated by the air-flow produced by the attack of a predator, this mechanosensitive system triggers jumping or running, to move away from the danger. The filiform wind-receptors are among the most sensitive animal mechanoreceptors. Cercal sensory inputs and motor units are usually connected by a reduced number of interneurons bearing axons of large diameters, allowing the information to be quickly transmitted to command units (Edwards and Palka, 1974; Palka et al., 1977; Camhi, 1980; Edwards and Williams, 1981; Ritzmann, 1984; Boyan and Ball, 1986, 1989).

The wind-activated escape system of crickets constitutes a classical model in neuroethology (e.g., Bacon and Murphey, 1984; Jacobs et al., 1986; Miller et al., 1991; Jacobs and Theunissen, 1996, 2000; Ogawa et al., 1999, 2004; Paydar et al., 1999; Dangles et al., 2006a,b). The most detailed description of the organization of the nervous elements associated to this system in any Orthoptera is the analysis by Seabrook (1970) of the terminal ganglionic mass of the locust, *Schistocerca gregaria*. Descriptions of the organization of sensory afferents and giant interneurons into the terminal abdominal ganglion are also available for the crickets *Acheta* and *Gryllus* and the cockroach *Periplaneta* (e.g., Edwards and Palka, 1974; Mendenhall and Murphey, 1974; Sasira Babu and Subhashini, 1981; Blagburn et al., 1984; Boyan et al., 1989; Blagburn and Thompson, 1990).

The knowledge gained recently on the wood cricket *Nemobius sylvestris* (Orthoptera: Gryllidae) offers a unique opportunity to integrate, for a single and same species, neuroethology in a natural context (Dangles et al., 2005) up to the physical basis of air-flow production and detection (Dangles et al., 2006a,b; Magal et al., 2006; Steinman et al., 2006). The wood-cricket, *N. sylvestris*, is a particularly suitable model for integrative physiology as its biology can be easily studied in the field in contrast to other cricket species classically investigated (e.g., Coolen et al., 2005; Steinmann et al., 2006; Dangles et al., 2005, 2006a,b). The comprehension of how air-currents are perceived and coded by the nervous system is relevant not only to understand the adaptive value and evolution of this kind of sensory system, but also as a source of inspiration for the development of new technologies, such as microelectrical–mechanical systems (MEMS, Dijkstra et al., 2005). The aim of the present work is thus to describe the gross anatomical structure of the terminal abdominal ganglion in the wood cricket and to establish a basis for subse-

Contract grant sponsor: European Community (Customized Intelligent Life Inspired Arrays, CILIA project); Contract grant number: FP6-IST-016039.

*Correspondence to: Teresita C. Insausti, Institut de Recherche sur la Biologie de l'Insecte, Université François Rabelais, Faculté des Sciences et Techniques, Avenue Monge, Parc Grandmont, 37200, Tours, France. E-mail: tere.insausti@univ-tours.fr

Published online in
Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10672

quent physiological studies both, in the laboratory and in the field.

MATERIALS AND METHODS

Animals

Adult crickets, *Nemobius sylvestris* (Bosc, 1792) of both sexes, sampled in surrounding woodland areas of Tours (France) and maintained in the laboratory during the winter months, were used throughout this study.

Afferent Staining

The projection of sensory neurons of cercal sensilla within the terminal ganglion was determined by backfilling with cobalt chloride ($CoCl_2$). The insects were immobilized ventral side up with double sided tape on a plastic Petri dish. In some cases, a single sensillum or group of sensilla was pulled out and a drop of 2.5% cobalt chloride placed on the sensillum base, which was then covered with Vaseline. In other cases, a portion of the cercal nerve was dissected out and cut, and the cut end was placed in 2.5% cobalt chloride for a mass backfilling. Preparations were left overnight at 10°C in a humid chamber or for 4–5 h at 21°C. The terminal ganglion was thereafter dissected out and the cobalt precipitated by treating the ganglion with a freshly prepared solution of ammonium sulfide in saline. The preparations were then fixed in glacial acetic acid/ethanol/formalin fixative and intensified with silver by Bacon and Altman's (1977) wholemount Timms's procedure. This was followed by dehydration, clearing in methyl salicylate, and mounting in Permount. The central arborization of successfully stained neurons was drawn from wholemounts and photographed.

The same kind of anterograde staining has been performed using fluorescent dyes. A solution of 1% Neurobiotin (Vector) in 0.25 M KCl was applied to the cercal nerve using the same procedure as described for cobalt fillings. The dissected ganglion was fixed overnight at room temperature in 4% paraformaldehyde in Milloning phosphate buffer at pH 7.2. The ganglion was dehydrated and rehydrated, and then incubated in a 0.025% phosphate buffered Oregon Green-avidin solution (NeutrAvidin, Oregon Green 488 conjugate, Molecular Probes, A6374) containing 1% bovine serum albumin and 0.25% Triton X-100 for 12 h at 4°C. The ganglion was then dehydrated, cleared in methyl salicylate, and mounted in Permount (Merck) as wholemount. The preparations were examined using an Olympus FluoView 500 confocal laser-scanning microscope, equipped with lasers: Ar 488 nm and 514 nm, and HeNe 544 nm and 633 nm. Stacks of optical sections were analyzed using ImageJ software.

Interneuron Staining

The giant ascending interneurons were stained with cobalt chloride by retrograde axonal diffusion. The ventral cuticle of the second abdominal segment was stripped away, exposing the abdominal ventral cord. The connectives anterior to the third abdominal ganglion were transected and a Vaseline well was built around, which isolated the ganglion from the abdominal nerve cord. A drop of 2.5% cobalt chloride was placed in the well, which was sealed over with Vaseline. The preparation was left at room temperature for 4–5 h or overnight at 10°C in a humid chamber. The procedure was then the same as that described previously.

Two different fluorescent dyes were used to label the giant interneurons and the sensory axons of the cerci simultaneously. A 5% dextran tetramethylrhodamine solution (Molecular Probes, D3308) was applied in the connectives using the same procedure as described for cobalt labeling, and neurobiotin-oregon green was applied to the cercal nerve using the same tracing technique as described earlier.

Light Microscopy

This was performed on crickets nervous system processed following the technique described by Ribi (1987). Briefly, the distal part of the abdomen was sectioned and then fixed for 3–4 h in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate buffer (pH 7.3) with sucrose and $CaCl_2$ added. Subsequently, the pieces were postfixed with buffered 1% osmium tetroxide for 1–2 h. After dehydration, they were embedded via propylene oxide in Durcupan. The blocks were serially sectioned at 2–5 μm thick using glass knives. The sections were stained on a hot plate with Toluidine Blue–Basic Fuchsin.

To elucidate nerve tracts and neuropilar areas within the terminal abdominal ganglion, a modification of the osmium tetroxide and ethyl gallate methods of fixing and staining described by Wigglesworth (1957) was used. Ganglia were removed from live animals, fixed for 3 h in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate buffer (pH 7.3) with sucrose and $CaCl_2$ added. They were then rinsed in buffer and postfixed with phosphate-buffered 1% osmium tetroxide in the dark at 4°C for 2 h on a rotator, and for an additional 30 min at room temperature. After another rinse in buffer, they were stained in fresh supersaturated ethyl gallate solution overnight at 4°C, dehydrated, and embedded in epoxy resin (Durcupan Fluka). Horizontal, transverse, and sagittal sections, 10 μm thick, were then cut, mounted with Permount, and examined with a light microscope. Photographs and drawings were made from appropriate sections.

Scanning Electron Microscopy

The sensilla canopy structure was examined by means of scanning electron microscopy (SEM, DSM 982 GEMINI, LEO Microscopie) of cerci that had been dissected from crickets, dehydrated, and sputter coated with platinum.

The terminology for the tracts of the terminal abdominal ganglion is based on that employed for locust by Tyrer and Gregory (1982). The giant interneurones are named according to Jacobs and Murphey (1987).

RESULTS

The Cerci

The cercal system comprises of the following: the external structures (cerci) with their complement of sensilla, the projections of sensory axons into the terminal ganglion of the ventral nervous chain, and the postsynaptic cells activated by cercal sensory inputs. The cerci of *Nemobius sylvestris* are covered by a variety of sensilla which differ in length, thickness, and type of socket. Five different types of sensilla have been identified. These are as follows: 1) filiform sensilla, 2) dome-shaped campaniform sensilla, 3) clavate sensilla, 4) long slender bristles, and 5) short bristles (Fig. 1A). The filiform sensilla in the crickets are distributed everywhere on the cercus. The cercal campaniform sensilla are combined with the sockets of filiform or clavate sensilla, usually in number of two per sensillum (Fig. 1A,D). Sensory axons arising from the cercal sensilla, whose somata lie at the hair base, assemble in groups that coalesce to form two bundles in the base of the cercus (Fig. 1B,D, cn). These bundles fuse to form the stout cercal sensory nerve (Fig. 1C, CN).

Fig. 1. The cercus of *Nemobius sylvestris*. **A**: The surface of an adult cercus. SEM. b, bristle sensillum; c, campaniform sensillum; cl, clavate sensillum; f, filiform sensillum. Scale bars: 200 μm. The inserts show details of a filiform sensillum and a campaniform sesillum (arrow) (top left), of bristle sensilla (bottom left) and of a clavate sensillum (bottom right). **B**: Transverse sections at the base of the cercus showing the two bundles of the cercal nerve (cn). LM. **C**: Transverse section of the cricket abdomen showing the stout bilateral nerves (CN) formed by the fusion of the two nervous bundles (cn in Fig. B) running into each cercus. LM. **D**: Section though the socket of a filiform sensillum (sf) and one of the members of the pair of related campaniform sensilla (c). LM. B, D: Toluidine Blue–Basic Fuchsin staining; D: Ethyl Gallate method. Scale bars: 20 μm

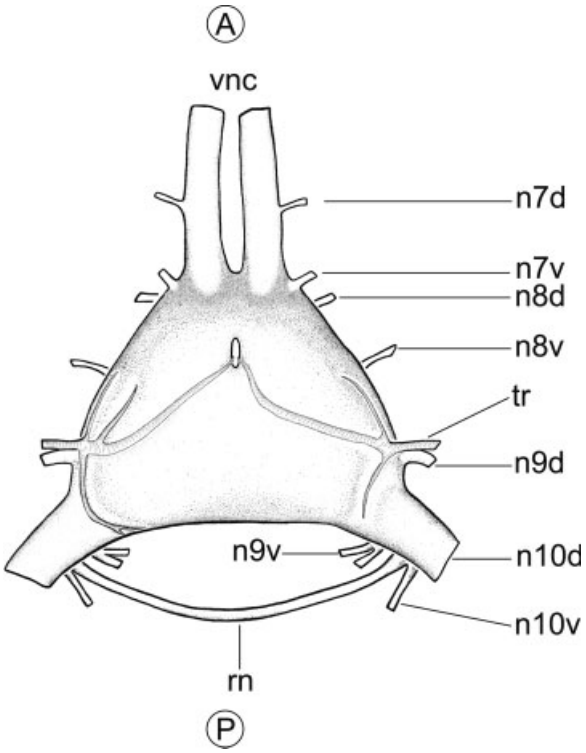

Fig. 2. Diagram of the terminal abdominal ganglion of *Nemobius sylvestris* in ventral view. The ganglion is the fusion of the ganglia belonging to the7th to 11th abdominal segments. The associated nerves (n7v–n10v, ventral nerves; n7d–n10d, dorsal nerves; rn, ring nerve), the tracheae (tr), and the ventral nerve cord (vnc) are shown. A, anterior; P, posterior.

The Central Nervous System

The gross anatomy of the central nervous system of *Nemobius sylvestris* is similar to that described for other crickets. It consists of a dorsal anterior brain, circumenteric connectives (that encircle the esophagus to unite ventral to the gut at the subesophageal ganglion), and a ventral nerve cord with segmental ganglia and segmental peripheral nerves. The ventral nerve cord includes three large segmental thoracic ganglia, one for each segment of the thorax, but the metathoracic ganglion also includes the ganglia of the first two abdominal segments. Five smaller ganglia are spaced along the nerve cord into the abdomen. The fifth one, the terminal abdominal ganglion, is formed by the complete fusion of the 7th to 11th segmental ganglia.

The Terminal Abdominal Ganglion

The terminal abdominal ganglion of *Nemobius sylvestris* has four paired ventral and dorsal nerves which innervates the abdominal segments 7 to 10. The cercal nerves (dorsal n10) contain the bundle of cercal sensory axons, which enter the posterolateral corners of the terminal ganglion (see Fig. 2).

The central core of the terminal abdominal ganglion is a matrix of neural processes designated as neuropils in the broad sense. Groups of fibers running together in bundles form the longitudinal tracts and the transverse tracts (commissures).

We have identified nine longitudinal tracts in the terminal abdominal ganglion of *Nemobius sylvestris*, two major tracts (LDT = lateral-dorsal-tract and VIT = ventral-intermediate-tract) and seven smaller tracts (DIT = dorsal-intermediate-tract, MDT = median-dorsal-tract, DMT = dorsal-median-tract, VMT = ventral-medial-tract, MVT = median-ventral-tract, VLT = ventral-lateral-tract and LVT = lateral-ventral-tract) (Figs. 3B–D and 4A,B). These tracts retain those positions only in the anterior region of the terminal abdominal ganglion. As they reach the posterior region, the fibers distribution begins to alter. There are also distinctive oblique tracts; the two most prominent in this category being related to segmental nerves 8v and 9d (Figs. 2, 4D).

The ganglion cell bodies are located at the periphery, in the lateral region of the anterior part of the terminal abdominal ganglion, and laterodorsal in the posterior part of the terminal abdominal ganglion. The dorsal and ventral central regions of the terminal abdominal ganglion are mostly free of somata (Fig. 3B,C).

The Giant Ascending Interneurons

We have identified a population of at least seven pairs of pear-shaped cell bodies of very large diameter (so-called giant interneurons), organized symmetrically on both sides of the ganglion (Figs. 3C and 5). In this cricket, the giant fiber population of the ventral nerve cord is composed of a group of seven axons with diameters comprising between 20 and 45 μm (measured at the interganglionar connective) (Fig. 3A). The neuropil of the ganglion is dominated by the processes of these giant interneurons, which lie in the two major tracts lateral–dorsal and ventral–intermediate. Four of the largest axons are located superficially in the lateral–dorsal tract, accompanied by a group of smaller fibers. The fifth largest axon is located in the ventral–intermediate tract together with the other two smaller giant interneurons and a group of fibers of small size (Figs. 3B–D and 4A,B). The tracts of giant interneurons are interconnected by two commissures. The anterior one is loosely organized. The posterior one has many fibers crossing the midline of the ganglion in close apposition (Figs. 3C and 4F). A glomerulus-like structure is present in the dense neuropil of the posterior region of the terminal abdominal ganglion. It receives the sensory fibers of cercal nerves and the terminal processes of giant interneurons (Fig. 4C,E).

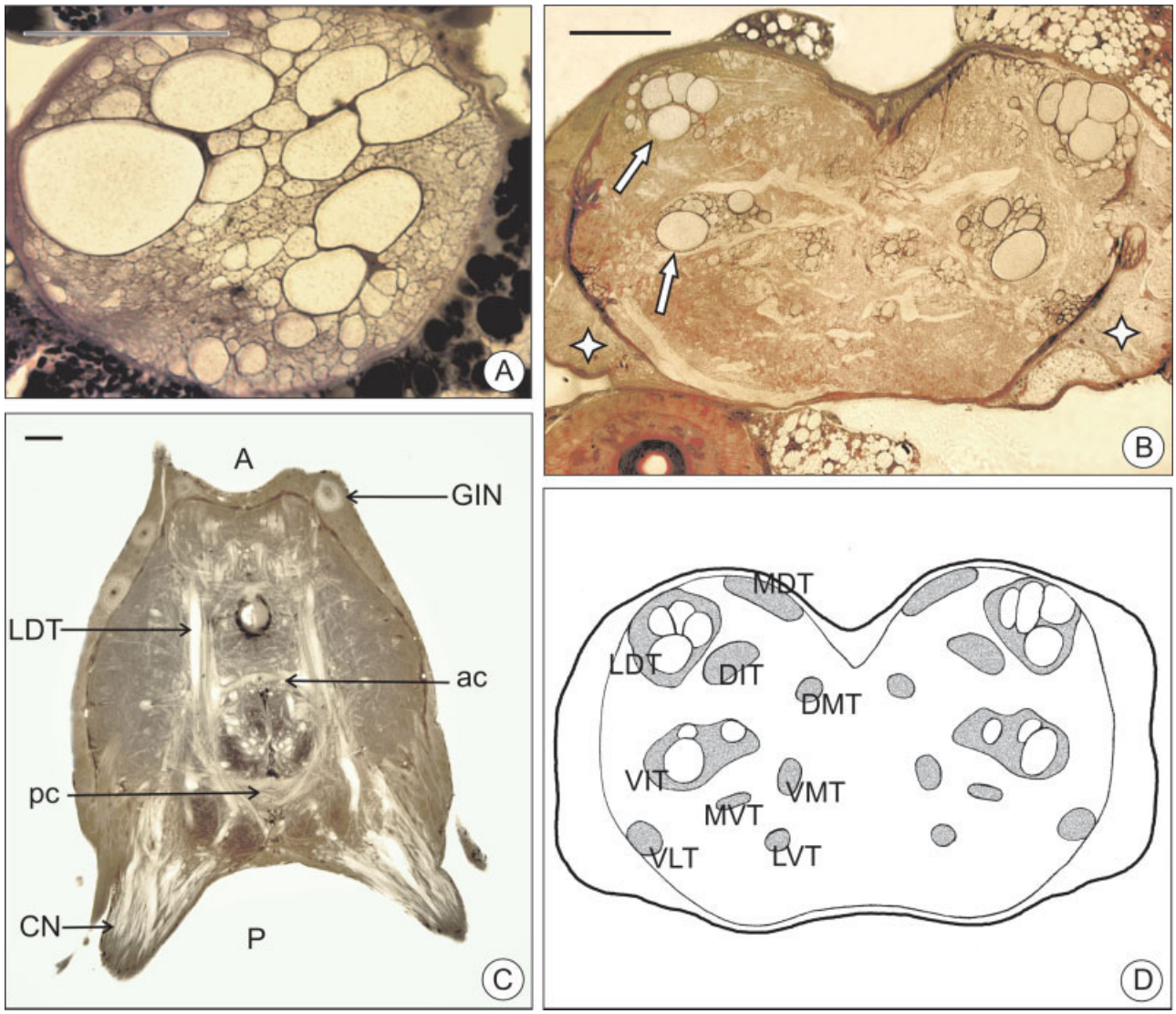

Fig. 3. **A**: A transverse section of one connective of the ventral nerve cord, close to the terminal abdominal ganglion. LM. **B**: Transverse section of the anterior region of the terminal abdominal ganglion; arrows indicate the two longitudinal tracts where the giant axons are located. The stars indicate the cell body regions at each side of the ganglion. LM. **C**: Horizontal section of the terminal abdominal ganglion, showing the giant cell bodies (GIN) located in the periphery of the ganglion, the tracts (LDT: lateral dorsal tract) and commissures (ac, anterior commissure; pc, posterior commissure), and the entrance of the cercal sensory axons via the cercal nerves (CN). LM. **D**: Schematic diagram of a transversal section of the terminal abdominal ganglion showing the tracts and the giant axons located in the lateral dorsal tract (LDT), and the ventral intermediate tract (VIT). A, anterior; P, posterior; DIT, dorsal intermediate tract; DMT, dorsal medial tract; LVT, lateral ventral tract; MDT, medial dorsal tract; MVT, medial ventral tract; VLT, ventral lateral tract; VMT, ventral medial tract. A, B: Toluidine Blue - Basic Fuchsin staining; C: Ethyl Gallate method. Scale bars: 50 μm.

Mass retrograde cobalt fills from connectives linking the terminal abdominal ganglion to the sixth abdominal ganglion confirmed the existence of the population of seven pairs of cells of very large diameters, organized symmetrically on both sides of the ganglion (see Fig. 5). Each giant interneuron is characterized by the position of its soma and by a specific branching pattern. This group of cells maintains a constant position in the terminal ganglion. The somata of all identified giant interneurons are located contralateral to their axons and are positioned sequentially along the periphery of the ganglion. The giant interneurons are named according to the number corresponding to the original segment of the fused ganglion (Fig. 5A–F). We have identified a cell whose body is located in the 7th segment, 7-1a, two cells located in the 8th segment, 8-1a and 8-2a, three cells located in the 9th segment, 9-2a, 9-1b, and 9-3a and a cell located in the 10th segment, 10-3a. Figure 6 depicts a schematic representation of the relative position of the giant

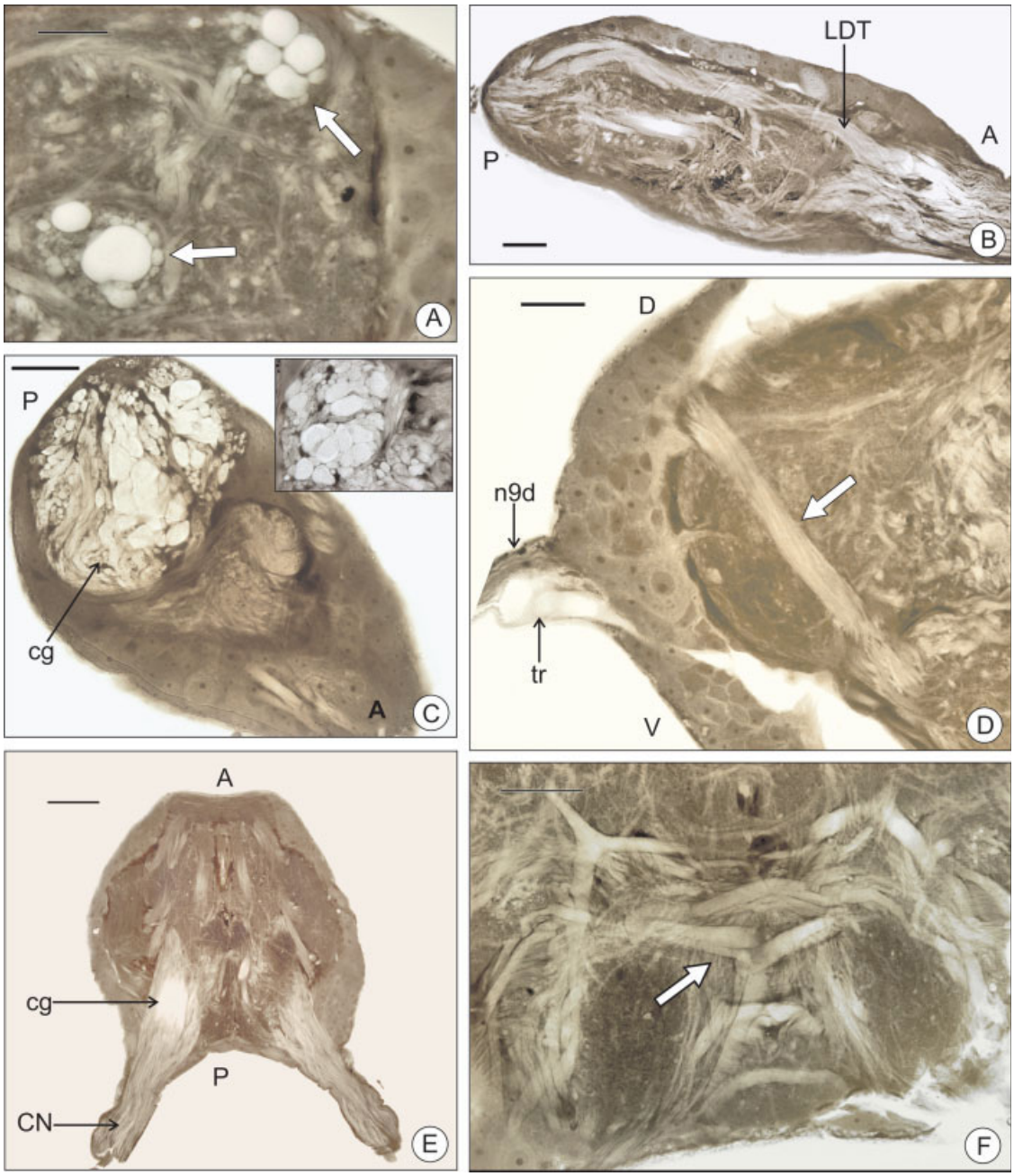

Fig. 4. *Nemobius sylvestris*. Light micrographs of sections of the terminal abdominal ganglion staining with Ethyl Gallate method. **A**: Detail of a transverse section of the terminal abdominal ganglion showing the two tracts where the giant interneurons axons run (arrows), LDT (right), and VIT (left). Four of the major axons are located in the LDT and the other three are located in the VIT. In both cases, they are surrounded by small fibers. Bundles of fibers connect both tracts between them. **B:** Sagittal section of the terminal abdominal ganglion where the pathway of the lateral dorsal tract is evident. **C:** Sagittal section of the terminal abdominal ganglion showing the cercal glomerular neuropil (cg); the insert shows a detail of the cercal glomerular neuropil as seen in a transverse section (scale bar: 20 μm). **D:** Transverse section of the terminal abdominal ganglion showing an oblique tract; the trachea (tr) and the nerve n9d can be seen. **E:** Section of the terminal abdominal ganglion showing the entrance of the sensory axons through the cercal nerves and the general aspect of the cercal neuropil (cg) in the horizontal plane. CN, cercal nerve. **F:** Detail of the posterior commissure in a horizontal section of the terminal abdominal ganglion; the crossing of the midline of the ganglion by the giant fibers is shown using an arrow. Scale bars: 50 μm. A, anterior; P, posterior.

interneurons into the terminal abdominal ganglion, compiled from series of sections of ethyl-gallate preparations in the three planes and several cobalt fillings. The most useful landmark for identifying the cells body position is the midline trachea which goes to center of the ganglion (Figs. 2, 4D, and 5E). Cells 9-1b and 9-2a are located near the arrival of the tracheae to the

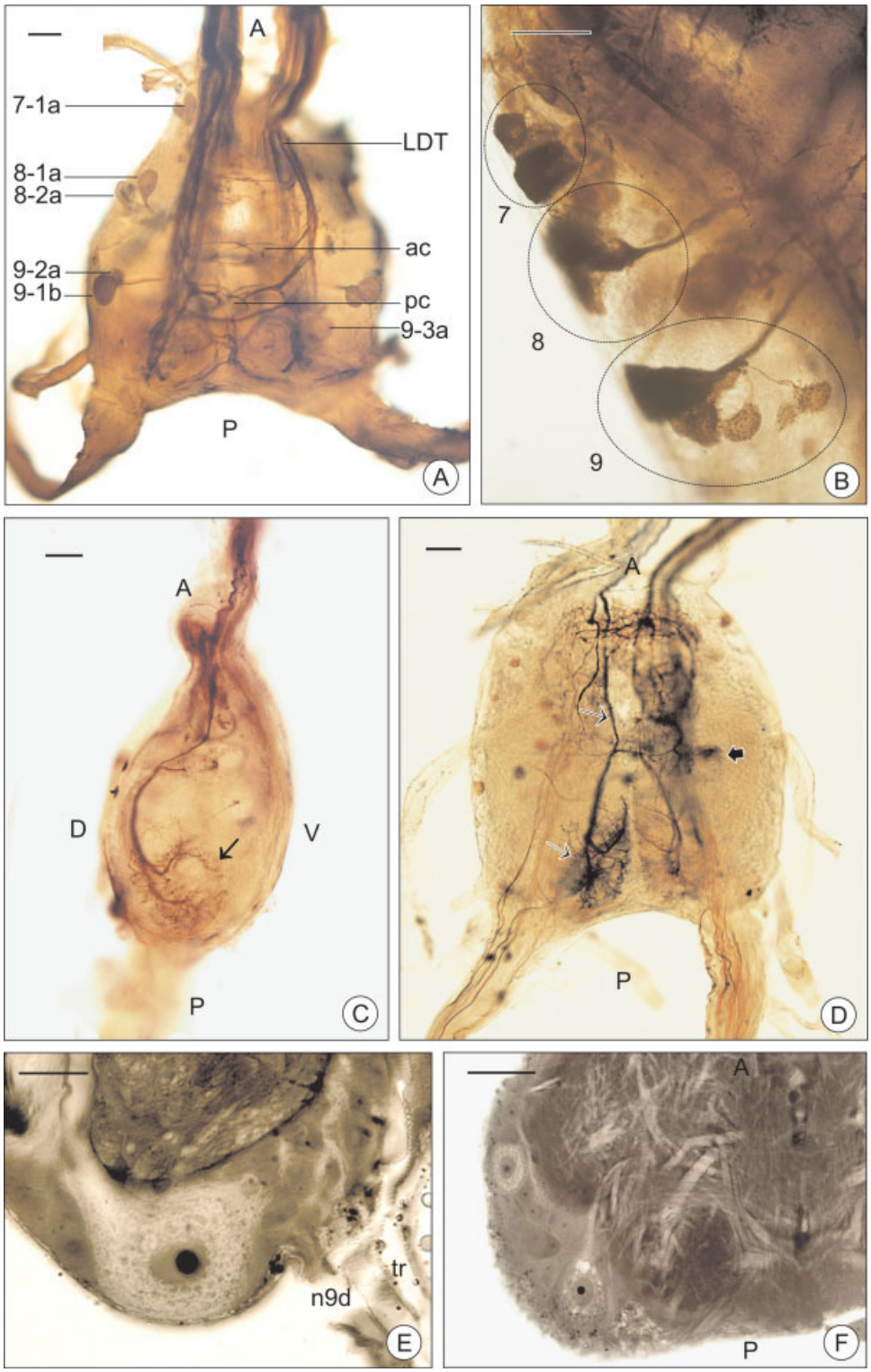

Fig. 5. *Nemobius sylvestris.* The terminal abdominal ganglion and the giant interneurons. LM. **A–D**: Wholemount preparation showing the location of the identified giant interneurons, as revealed by cobalt backfills from connectives (scale bars: 50 μm). Cells in A and B are labeled following the nomenclature proposed by Jacobs and Murphey (1987), by ascribing them to the neuromeres corresponding to the 7th to 10th abdominal segments. The lateral–dorsal tract is evinced in A (in the plane of focus). The segmental organization of giant interneurons can be seen in A and B. **B:** Detail of the region of the terminal abdominal ganglion to show cell clusters corresponding to neuromeres 7–9. Each cluster has a different number of giant interneurons. **C:** Lateral view of the terminal abdominal ganglion revealing the complete pathway of the fibers belonging to the giant interneurons 9-1b into the ganglion. The arrow indicates the arborization in the cercal glomerulus. **D**: Same preparation as C, but seen dorsally; the extension of the bilateral branching of the giant interneurons 9-1b can be appreciated. The short arrow indicates the cell body positioned contralateral to the axon. **E**: The soma of the cell 9-1b as seen in ethyl-gallate sections of the terminal abdominal ganglion. tr, tracheae. (scale bar: 20 μm). **F:** Terminal abdominal ganglion horizontal section to show the body of the cell 9-3a and its fiber entering the neuropil (scale bar: 50 μm). A, anterior; D, dorsal; P, posterior; V, ventral.

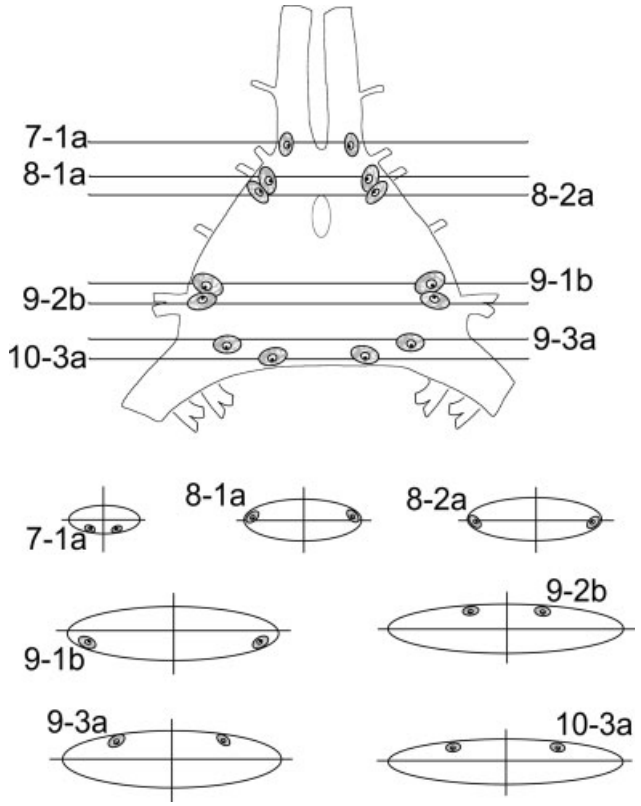

Fig. 6. Schematic representation of the terminal abdominal ganglion and the positions of the giant interneurons as reconstructed from several cobalt filling and serial sections of ethylgallate preparations in the three planes. The giant neurons of *Nemobius sylvestris* are labeled following the nomenclature proposed by Jacobs and Murphey (1987), by ascribing them to the neuromeres corresponding to the 7th to 10th abdominal segments.

ganglion together with nerve 9d. Groups 7 and 8 are located anteriorly and group 10 posteriorly.

The Projection of Sensory Axons

Mass cobalt and fluorescent dye fillings from the cercus were performed to identify the location of the sensory axons within the terminal abdominal ganglion. Two well-defined neuropil-like areas at each side of the terminal abdominal ganglion midline are revealed. All the fibers from the cercal nerve that synapse in the terminal ganglion appear to do so on the ipsilateral side, where they are often seen to contact branches of giant interneurons. Furthermore, two prominent areas of stout submedian contralateral projections are evident (Fig. 7A,C). Axons from certain cercal sensilla ascend through the connectives to reach, at least, the metathoracic ganglion (Fig. 7A–C). As they pass through each segmental abdominal ganglion, they arborize, sending branches into the medial region (Fig. 7E).

Anterograde selective fillings of a reduced number of wind sensitive filiform sensilla revealed arborization into the posterior neuropil. The afferent terminals outline glomerular masses of fibers on its whole surface (Figs. 7C,D, and 8).

DISCUSSION

The cercal system seems to have evolved with the first terrestrial hexapods, reaching its zenith in the orthopteroid insects. This system for evading predators seems to have been replaced in some insect groups, such Hemiptera and holometabola, by visual startle mechanisms with the same function (Edwards and Reddy, 1986). The stimulation of cercal wind-receptors triggers different motor patterns according to the behavioral context, that is, walking, flying, and swimming, and different escape strategies are executed, even in closely related species (Hirota et al., 1993; Matsuda et al., 2002; Kanou et al., 2006).

The Arrangement of Sensory Axons in the Terminal Abdominal Ganglion

The anatomical organization of the terminal abdominal ganglion of *Nemobius sylvestris* shares some characteristics with that of other cricket species, but differences also occur. Two neuropilar areas were revealed on each side of the terminal ganglion of *N. sylvestris* by means of massive injections of dyes through the cercal nerve and by histology. The largest area corresponds to the posterior cercal glomerulus and the smaller anterior region to the bristle neuropil of other cricket species (Murphey, 1981, 1985; Bacon and Murphey, 1984). Cercal filiform sensilla of *N. sylvestris* arborize into the first one. The selective injection of the dye into sensory neurons of the filiform sensilla revealed that they arborize over the whole surface of the cercal glomerulus, outlining it. In *Acheta domesticus*, there are two principal planes of insertion for the filiform hair, one allowing vibration on the longitudinal cercal axis on wind movement and another transversely (Edwards and Palka, 1974; Bacon and Murphey, 1984). In this species, each physiological type of sensillum arborizes in a different region of the cercal glomerulus (Bacon and Murphey, 1984). Further studies would be necessary to verify whether this is the case of *N. sylvestris*, or sensory inputs have a different arrangement. Murphey (1985) described in *A. domesticus* a system of hair-like receptors distributed over the cercus that he named "bristles." Sensory neurons from bristles project into a common area of the terminal abdominal ganglion (segments 7 and 8) that this author describes as the "bristle neuropil." Collaterals of several of these bristles leave the terminal abdominal ganglion to arborize on the ventral region of every abdominal segment. Furthermore, Heusslein and Gnatzy (1987) showed that in *Gryllus bimaculatus, A. domesticus,* and *Periplaneta americana,* the sen-

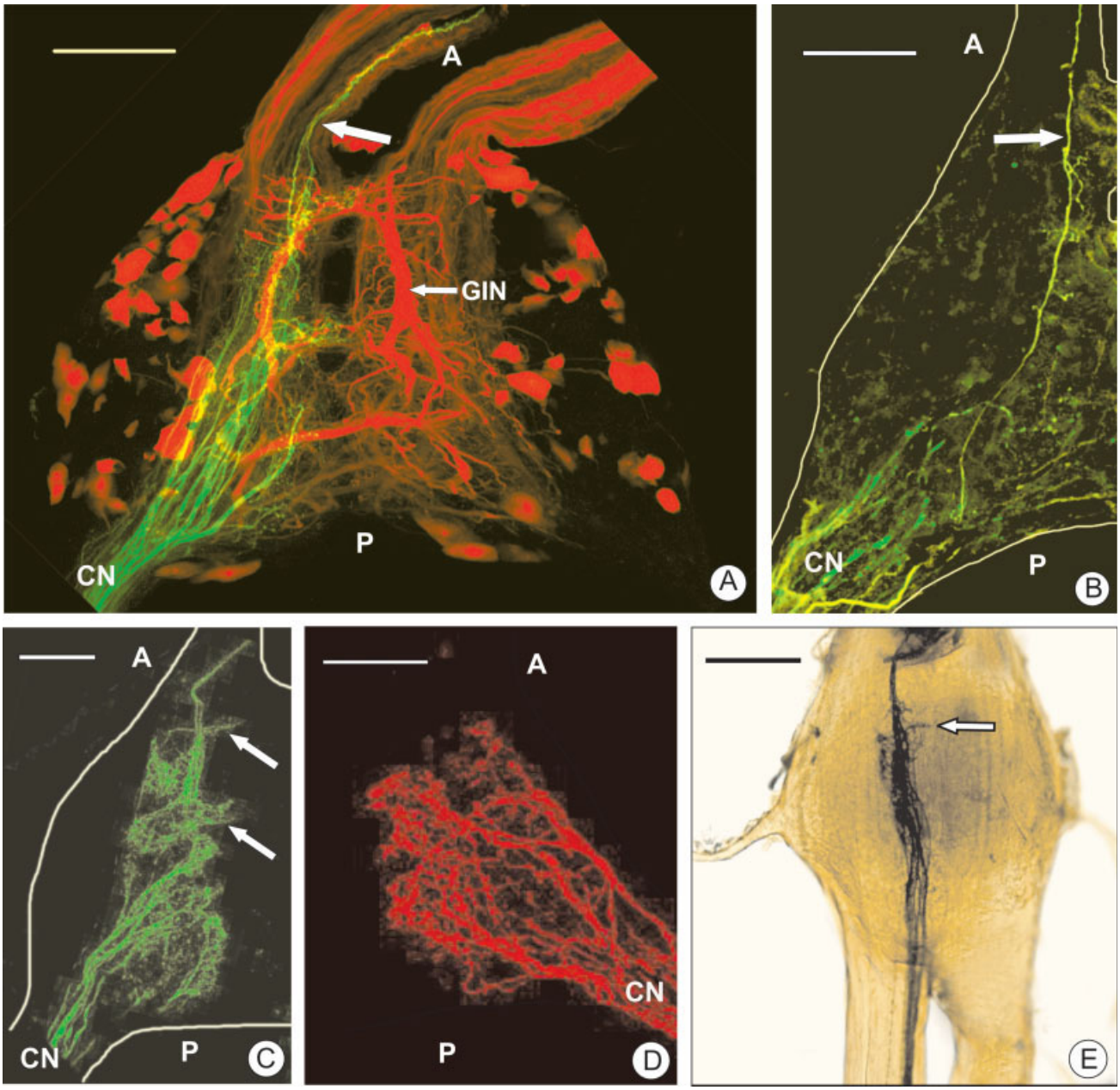

Fig. 7. *Nemobius sylvestris.* Anterograde and retrograde fills through cercal nerves (CN) and connectives, respectively. **A**: Confocal image of a wholemount preparation by double filling of sensory axons and ascending interneurons with fluorescent dyes. Some sensory axons (green) cross the terminal abdominal ganglion to continue ascending through the ventral nerve cord (arrow). Other axons contact in the terminal abdominal ganglion the branches of giant ascending interneurons (red). **B:** Confocal section showing an ascending sensory axon (arrow). **C:** Confocal section of a massive anterograde fill through the cercal nerve revealing sensory axons projecting into two different ipsilateral neuropil areas of the terminal abdominal ganglion. Arrows indicate two groups of axons projecting contralaterally. **D:** Confocal section of the ventral region of a cercal glomerulus showing the ending of axons belonging to filiform sensilla. **E**: Fourth abdominal ganglion, showing the pathway of ascending sensory axons, which send branches into the neuropil, as revealed by anterograde filling with cobalt chloride. Scale bars: 100 μm. A, anterior; CN, cercal nerve; GIN, giant interneuron; P, posterior.

sory axons of campaniform sensilla associated with filiform sensilla also ascend as "through fibers" from the terminal abdominal ganglion to reach the sixth abdominal ganglion. In *N. sylvestris*, a high number of thin fibers ascend from the terminal abdominal ganglion via the connectives, arborize in each abdominal segmental ganglion, and finally reach the metathoracic fused mass. Although the occurrence of ascending sensory fibers was therefore confirmed in our species by retrograde injection, our results do not allow us to elucidate which type of receptor ascends, at least, up to the thorax.

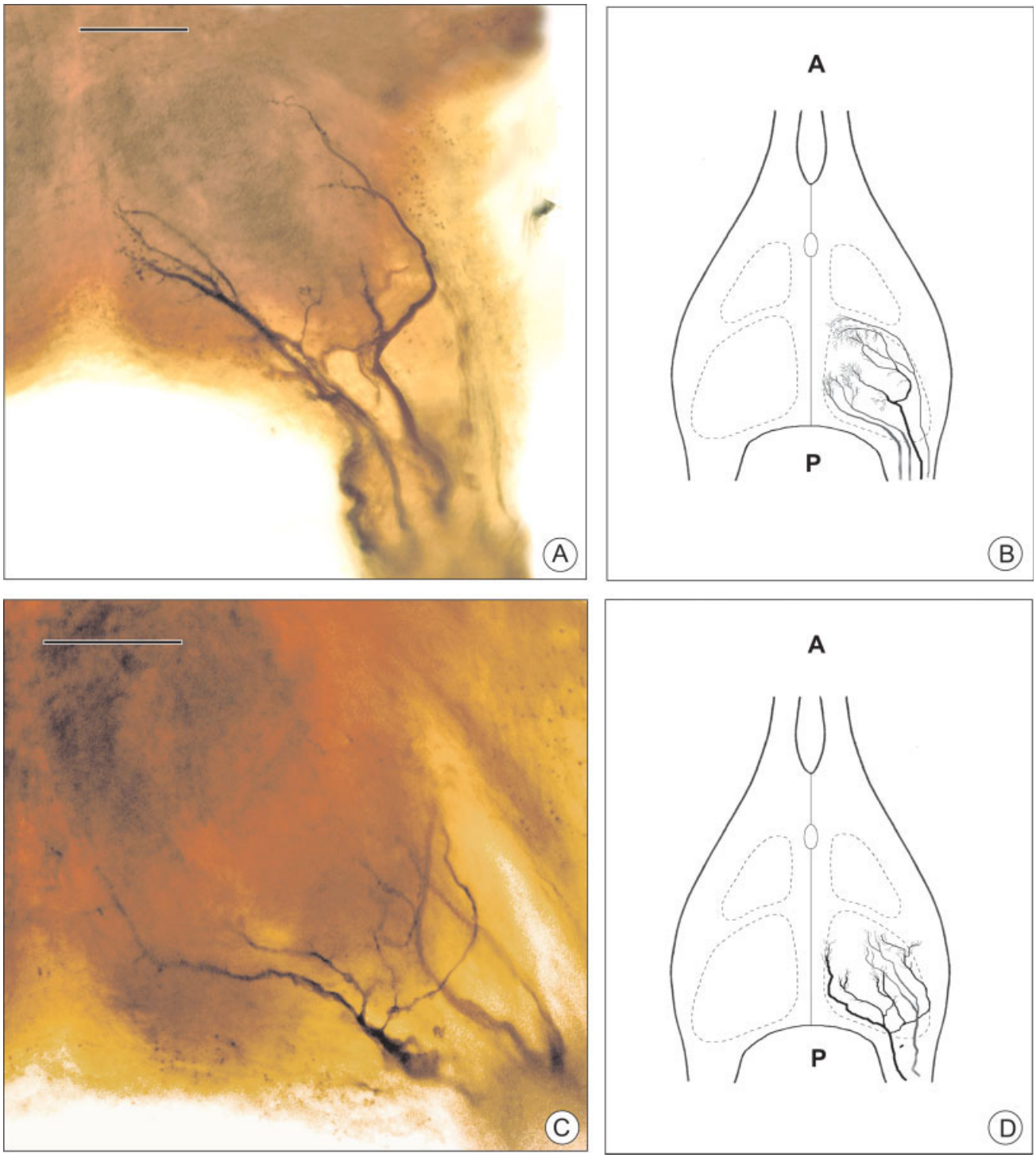

Fig. 8. *Nemobius sylvestris.* Selective anterograde cobalt chloride fills through a few filiform sensilla. **A**,**C:** Photomicrographs of wholemount preparations. **B**,**D:** Camera-lucida drawings showing the branching of individual sensory axons. Dorsal branches are indicated in black and ventral ones in gray. Sensory axons of filiform sensilla surround the wind-receptive cercal neuropil. Scale bars: 50 μm. A, anterior; P, posterior.

Sensory axons of the adult *Acheta domesticus* project only in the ipsilateral neuropils of the terminal abdominal ganglion, no contralateral terminations being observed (Edwards and Palka, 1974). Thus, bilateral interactions are mediated by interneurons and not by direct sensory inputs (Palka and Olberg, 1977). In *Nemobius sylvestris*, we have found two groups of contralateral projec-

tions in the anterior and median region of the terminal abdominal ganglion (Fig. 7A). This pattern has also been shown in *Grylloblatta* (three areas) and in the praying mantid *Archimantis brunneriana* (two projections) by Edwards and Mann (1981) and by Ball et al. (1982), respectively. According to the last authors, mantids also evince smaller and more sharply defined neuropil than that of crickets. Indeed, Edwards and Palka (1974) described glomerular neuropils of *A. domesticus* as loosely organized. Nevertheless, neuropils of *N. sylvestris* appear as quite compact, resembling those of mantids, rather than those of other cricket species. In addition, the number of sensory axons ascending through the nerve cord seems to be higher in mantids than in *A. domesticus* (Ball et al., 1982). In *N. sylvestris*, as mentioned earlier, an important number of sensory axons projects beyond the terminal abdominal ganglion. Nevertheless, we lack data concerning the other species to allow precise comparisons.

Segmental Homologies of Giant Interneurons

Because the early patterning of neurogenesis is similar in different segments of the same insect and is even conserved among different insects, it is sometimes possible to recognize homologous neurons in different ganglia and even in different insect species despite differences arising postembryonically as a consequence of functional differentiation. For example, specific interneurons in different neuromeres that have similar arrangement of their axons and arborization develop from precisely homologous cells during early development (Chapman, 1998). The giant neurons of *Nemobius sylvestris* can be ascribed to neuromeres corresponding to the 7th to 10th abdominal segments, based both on the comparative analysis with other cricket species and their position in the terminal abdominal ganglion. The somata of giant interneurons of *N. sylvestris* are positioned sequentially along the periphery of the ganglion. The *Acheta domesticus* giant interneurons retain the original segmental position into the fused neuromeres that form the terminal abdominal ganglion, according to Mendenhall and Murphey (1974). Jacobs and Murphey (1987) identified some of the giant interneurons of adult *Gryllus assimilus* and *A. domesticus* in the embryo, confirming this hypothesis. Thus, we can conclude that the occurrence and segmental position of giant interneurons are highly conserved characteristics among the different cricket species. The reason why different segments kept different numbers of giant interneurons (1, 2, 3, and 1 giant interneurons at each side in the 7th, 8th, and 9th segment, respectively) nevertheless remains to be clarified. Either only some cells of each segment differentiate in a giant one or the original number is equal along segments at the beginning, but subsequently undergoes a reduction in certain segments due to cellular death. The available information does not allow us to decide between these two possibilities.

The Giant Interneuron Fibers

In contrast to the very small diameters of most neurons, some insects have "giant" axons of varying size. These fibers can be descending, as in *Musca*, *Calliphora,* and *Drosophila* (Bacon and Strausfeld, 1986) or ascending units, as in cockroaches and crickets. According to Edwards and Palka (1974), 10 giant axons can be seen in the ventral nerve chain of the terminal abdominal ganglion of *Acheta domesticus*. In *Nemobius sylvestris,* seven pairs of giant interneurons, with their corresponding axons, leave the terminal abdominal ganglion to ascend though the ventral cord. In *Gryllus assimilus* and *A. domesticus*, axons of giant interneurons are distributed into two (dorsal–intermediate and ventral–intermediate; Jacobs and Murphey, 1987) or three (dorsolateral, ventromedial, and ventrolateral; Mendenhall and Murphey, 1974) longitudinal tracts. In *N. sylvestris,* they clearly occupy two tracts (lateral–dorsal and ventral–intermediate). Thus, the number and pathway organization of giant interneurons seem to vary among cricket species.

Considerations on Giant Interneurons Size

The giant fibers are not the largest caliber axons. They are considered as giant if they are discontinuously larger than the next largest fibers in that species, the absolute diameter not being considered crucial (Bullock and Horridge, 1965). The axon diameter of the giant interneurons varies according to species, being 20–60 μm in *Periplaneta americana*, 8–15 μm in *Locusta migratoria,* 12–16 μm in *Anax spp*., and 5–15 μm in *Tettigonia cantans* (Bullock and Horridge, 1965; Shen, 1983; Nation, 2002). In *Nemobius sylvestris*, the seven pairs of neurons here considered as giant interneurons possess axons of diameters ranging between 20 and 45 μm. This is larger than the diameters of axons in other cricket species of bigger size (e.g., *Acheta domesticus* = body size 16–20 mm, giant axons 10–40 μm diameter against 7–10 mm, and 20–45 μm in *N. sylvestris*). Two questions arise from this comparison. The first concerns the subjective criterion to consider a cell as giant or nongiant. Here, we applied a quite conservative criterion, following Edwards and Palka (1974), but no operational or statistical definition of "giant," relative to the diameter of other axons in the nervous system, has been so far proposed. As can be noted in Figure 3A, several other smaller fibers, not considered as giant interneurons here, appear much larger than most axons of the nervous sys-

tem. Therefore, the giant interneurons described here represent only "the quickest among the quickest" ascending mechanosensory pathways. The second question is that of the ratio between giant interneurons and body size. Provided that the velocity of conduction is directly proportional to the diameter of the axons, the escape reaction might be quicker in *N. sylvestris* than in other crickets for two reasons: the axons have larger diameters and ganglia are closer. Further electrophysiological work in our laboratory should raise some light on this question. This article provides the neuroanatomical framework for such physiological studies.

We have described the organization of the sensory inputs and ascending outputs of the terminal abdominal ganglion of *Nemobius sylvestris*, the only cricket species where sensory and behavioral traits are being integrated in an ecological context (Dangles et al., 2006a). Even when the neuroanatomical organization of the cercal mechanosensory system was revealed as being rather similar to that of other cricket species, our study was necessary to provide a solid basis for physiological or modeling work. Even when some cricket species, such as *Acheta domesticus* and *Grillus bimaculatus*, provided much information about the neural organization of the mechanosensory cercal system (e.g., Edwards and Palka, 1974; Mendenhall and Murphey, 1974; Bacon and Murphey, 1984; Jacobs et al., 1986; Miller et al., 1991; Jacobs and Theunissen, 1996, 2000; Ogawa et al., 1999, 2004; Paydar et al., 1999), this information needed to be verified for their validity concerning the wood cricket. As indicated earlier, *Nemobius sylvestris* is a smaller species as compared with *Acheta domesticus* and *Grillus bimaculatus* and this difference could also be reflected in the organization of the mechanosensory system. This showed not to be the case, but it was impossible *a priori* to know the answer. The main reason is that the selective pressures responsible for modeling the wind-sensitive cercal system could have been very different. Even when only fragmentary information is available, the mechanosensory system of *Acheta domesticus* has been ascribed to the detection of the attack of parasitoids (Gnatzy and Kämper, 1990; Gnatzy, 1996). In the case of *Nemobius sylvestris*, it has been recently shown that wolf spiders constitute the main predators in the natural habitat of this species (Coolen et al., 2005; Dangles et al., 2006b). The cercal air-flow detecting system, to be efficient, should be able to recover the relevant parameters of the air that is moving sensory hairs, to evaluate the predator position and the attack speed, as each predator produces a different flow field around their body according to their body size, form, and movement (Casas et al., 2008). It is surprising that different cricket species exhibiting different body sizes and submitted to different predators have conserved the same organization in their neural system. The conservation of the sensory and neural organization of this system reveals it as a robust solution to analyze sensory information that may vary considerably in terms of the form of the flow field.

ACKNOWLEDGMENTS

The authors wish to thank Fabrice Vannier for assistance with the SEM and confocal microscope.

LITERATURE CITED

Bacon JP, Altman JS. 1977. A silver intensification method for cobalt-filled neurons in wholemount preparations. Brain Res 138:359–363.

Bacon JP, Murphey RK. 1984. Receptive fields of cricket (*Acheta domesticus*) interneurons are related to their dendritic structure. J Physiol 352:601–623.

Bacon JP, Strausfeld NJ. 1986. The dipteran 'Giant fibre' pathway: Neurons and signals. J Comp Physiol A 158:529–548.

Ball EE, Boyan GS, Stone RC. 1982. The cercal receptor system of the praying mantid, *Arcimantis brunneriana* Sauss. II. Cercal nerve structure and projection and electrophysiological responses of individual receptor. Cell Tissue Res 224:71–80.

Blagburn JM, Thompson KSJ. 1990. Specificity of filiform hair afferent synapses onto giant interneurons in *Periplaneta americana*: Anatomy is not a sufficient determinant. J Comp Neurol 302:255–271.

Blagburn JM, Beadle DJ, Sattelle DB. 1984. Synapses between an identified giant interneurone and a filiform hair sensory neurone in the terminal ganglion of first instar cockroaches (*Periplaneta americana L.*). J Exp Biol 113:477–481.

Boyan GS, Ball EE. 1986. Wind-sensitive interneurones in the terminal ganglion of praying mantids. J Comp Physiol A 159:773–789.

Boyan GS, Ball EE. 1989. The wind-sensitive cercal receptor/giant interneurone system of the locust, *Locusta migratoria*. II. Physiology of giant interneurones. J Comp Physiol A 165:511–521.

Boyan GS, Williams JLD, Ball EE. 1989. The wind-sensitive cercal receptor: Giant/interneurone system of the locust, *Locusta migratoria*. I. Anatomy of the system. J Comp Physiol A 165:495–510.

Bullock TH, Horridge GA. 1965. Structure and Function in the Invertebrate Nervous System. San Francisco, London: W.H. Freeman & Co. 1719 p.

Camhi JM. 1980. The escape system of the cockroach. Sci Am 243:144–157.

Casas J, Steinmann T, Dangles O. 2008. The aerodynamic signature of running spiders. PLoS ONE 3:e2116.

Chapman RF. 1998. The Insects. Structure and Function. United Kingdom: Cambridge University Press. 770 p.

Coolen I, Dangles O, Casas J. 2005. Social learning in non colonial insects? Curr Biol 15:1931–1935.

Dangles O, Magal C, Pierre D, Olivier A, Casas J. 2005. Variation in morphology and performance of predator-sensing system in wild cricket populations. J Exp Biol 208:461–468.

Dangles O, Casas J, Coolen I. 2006a. Textbook cricket goes to the field: The ecological scene of the neuroethological play. J Exp Biol 209:393–398.

Dangles O, Ory O, Steinmann T, Christides JP, Casas J. 2006b. Spider's attack vs. cricket's escape: Velocity modes determine success. Anim Behav 72:603–610.

Dijkstra M, van Baar J, Wiegerink R, Lammerink T, de Boer J, Krijnen G. 2005. Artificial sensory hairs based on the flow sensitive receptor hairs of crickets. J Micromech Microengineer 15:132–138.

Edwards JS, Mann D. 1981. The structure of the cercal sensory system and ventral nerve cord of *Grylloblatta.*. A comparative study. Cell Tissue Res 217:177–188.

Edwards JS, Palka J. 1974. The cerci and abdominal giant fibers of the house cricket *Acheta domesticus*. I. Anatomy and physiology of normal adults. Proc R Soc Lond B 185:83–103.

Edwards JS, Reddy GR. 1986. Mechanosensory appendages and giant interneurons in the firebrat (*Thermobia domestica*, Thysanura): A prototype system for terrestrial predator evasion. J Comp Neurol 243:535–546.

Edwards JS, Williams L. 1981. Anterior-most projections of giant interneurones in *Acheta domesticus* terminate in mechano-receptor neuropil of the brain. Soc Neurosci Abs 7:252.

Gnatzy W. 1996. Digger wasp vs. cricket: Neuroethology of a predator–prey interaction. In: Lindauer M, editor. Information Processing in Animals, Vol. 10. Stuttgart: Fisher-Verlag. pp 1–92.

Gnatzy W, Kämper G. 1990. Digger wasp against cricket. II. A signal produced by a running predator. J Comp Physiol A 167:551–556.

Heusslein R, Gnatzy W. 1987. Central projections of campaniform sensilla on the cerci of crickets and cockroaches. Cell Tissue Res 247:591–598.

Hirota K, Sonoda Y, Baba Y, Yamaguchi T. 1993. Distinction in morphology and behavioral role between dorsal and ventral groups of cricket giant interneurons. Zool Sci 10:705–709.

Jacobs GA, Murphey RK. 1987. Segmental origins of the cricket giant interneuron system. J Comp Neurol 256:145–157.

Jacobs GA, Theunissen FE. 1996. Functional organization of a neural map in the cricket cercal sensory system. J Neurosci 16:769–784.

Jacobs GA, Theunissen FE. 2000. Extraction of sensory parameters from a neural map by primary sensory interneurons. J Neurosci 20:2934–2943.

Jacobs GA, Miller JP, Murphey RK. 1986. Integrative mechanisms controlling directional sensitivity of an identified sensory interneuron. J Neurosci 6:2298–2311.

Shen J-X. 1983. The cercus-to-giant interneuron system in the bushcricket *Tettigonia cantans*: Morphology and response to low-frequency sound. J Comp Physiol A 151:449–459.

Kanou M, Nawae M, Kuroishi H. 2006. Cercal sensory system and giant interneurons in *Gryllodes sigillatus*. Zool Sci 23:365–373.

Magal C, Dangles O, Caparroy P, Casas J. 2006. Hair canopy of cricket sensory system tuned to predator signals. J Theor Biol 241:459–466.

Matsuda T, Kanou M, Yamaguchi T. 2002. Motor program initiation and selection in crickets, with special reference to swimming and flying behaviour. J Comp Physiol A 187:987–995.

Mendenhall B, Murphey RK. 1974. The morphology of cricket giant interneurons. J Neurobiol 5:565–580.

Miller JP, Jacobs GA, Theunissen,FE. 1991. Representation of sensory information in the cricket cercal sensory system. I. Response properties of the primary interneurons. J Neurophysiol 66:1680–1689.

Murphey RK. 1981. The structure and development of a somatotopic map in crickets: The cercal afferent projection. Dev Biol 88:236–246.

Murphey RK. 1985. A second cricket sensory system: Bristle hairs and the interneurons they activate. J Comp Physiol A 156:357–367.

Nation JL. 2002. Insect Physiology and Biochemistry. Boca Raton: CRC Press.

Ogawa H, Baba Y, Oka K. 1999. Dendritic Ca^{2+} transient increase evoked by wind stimulus in the cricket giant interneuron. Neurosci Lett 275:61–64.

Ogawa H, Baba Y, Oka K. 2004. Directional sensitivity of dendritic calcium responses to wind stimuli in the cricket giant interneuron. Neurosci Lett 358:185–188.

Palka J, Olberg R. 1977. The cercus-to-giant interneuron system of crickets. III. Receptive field organization. J Comp Physiol 119:301–317.

Palka J, Levine R, Schubiger M. 1977. The cercus-to-giant interneuron system of crickets. I. Some attributes of the sensory cells. J Comp Physiol 119:267–283.

Paydar S, Doan CA, Jacobs GA. 1999. Neural mapping of direction and frequency in the cricket cercal system. J Neurosci 19:1771–1781.

Ribi WA. 1987. Biological Electron Microscopy. A Handbook in Biological Electron Microscopy. Germany: Max-Planck-Institüt und Universität Tübingen.106 p.

Ritzmann RE. 1984. The cockroach escape response. In: Eaton RC, editor. Neural Mechanisms of Startle Behavior. New York: Plenum Publishing Corporation. pp 93–131.

Sasira Babu K, Subhashini K. 1981. Morphology of soma and dendrites of the giant fiber system in the sixth abdominal ganglion of the cockroach. J Morphol 169:351–355.

Seabrook WD. 1970. The structure of the terminal ganglionic mass of locust, *Schistocerca gregaria* (Forskäl). J Comp Neurol 138:63–86.

Steinmann T, Casas J, Krijnen G, Dangles O. 2006. Air-flow sensitive hairs: Boundary layers in oscillatory flows around arthropod appendages. J Exp Biol 209:4398–4408.

Tyrer NM, Gregory GE. 1982. A guide to the neuroanatomy of locust subesophageal and thoracic ganglia. Philos Trans R Soc Lond B 297:91–124.

Wigglesworth VB. 1957. The use of osmium in the fixation and staining of tissues. Proc R Soc Lond B 147:185–199.