Tissue and Cell 43 (2011) 52–65

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

The morphology and fine structure of the giant interneurons of the wood cricket *Nemobius sylvestris*

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ARTICLE INFO

Article history:
Received 5 May 2010
Received in revised form
30 November 2010
Accepted 4 December 2010
Available online 8 January 2011

Keywords:
Cricket cercal system
Neuroanatomy
Fine structure
Giant interneurons
Terminal abdominal ganglion

ABSTRACT

The structural and ultrastructural characteristics of giant interneurons in the terminal abdominal ganglion of the cricket *Nemobius sylvestris* were investigated by means of cobalt and fluorescent dye backfilling and transmission electron microscopy.

The projections of the 8 eight pairs of the biggest ascending interneurons (giant interneurons) are described in detail. The somata of all interneurons analyzed are located contralateral to their axons, which project to the posterior region of the terminal ganglion and arborise in the cercal glomerulus. Neuron 7-1a is an exception, because its arborisation is restricted to the anterior region of the ganglion. The fine structure of giant interneurons shows typical features of highly active cells. We observed striking indentations in the perineural layer, enabling the somata of the giant interneurons to be very close to the haemolymph. The cercal glomerulus exhibits a high diversity of synaptic contacts (i.e. axo-dendritic, axo-axonic, dendro-axonic, and dendro-dendritic), as well as areas of tight junctions. Electrical synapses seem to be present, as well as mixed synapses. The anatomical organization of the giant interneurons is finally discussed in terms of functional implications and on a comparative basis.

1. Introduction

Many Orthopteroid insects posses a pair of abdominal appendages, the cerci, involved in the detection of air-movements. They mediate the detection of the air stream preceding an approaching predator, triggering escape behaviour. Due to its relative simplicity, the wind-activated escape system of crickets has been intensively studied, representing a classical model-system for neuroethologists (e.g., Edwards and Palka, 1974; Palka et al., 1977; Edwards and Williams, 1981; 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 cercal system of crickets comprises the support structures (cerci), sensory hairs, projections of sensory axons into the terminal ganglion of the central nervous system and postsynaptic cells activated by cercal sensory input. The cerci are covered by a variety of hairs which differ in length, thickness and articulation (Sihler, 1924; Schmidt and Gnatzy, 1971; Gnatzy and Schmidt, 1972). Sensitivity to air-currents is mainly accomplished by mechanoreceptive filiform hairs, i.e., long thin hairs attached to a wide socket usually associated to two campaniform sensilla. Sensory neurons of filiform hairs project into the terminal abdominal ganglion, where they synapse with local and ascending interneurons, mainly giant interneurons. These quickly transmit the information to command units located in superior centres (Edwards and Palka, 1974; Palka et al., 1977; Camhi, 1980; Gnatzy and Tautz, 1980; Edwards and Williams, 1981; Ritzmann, 1984; Boyan and Ball, 1986, 1989; Gnatzy and Kämper, 1990; Insausti et al., 2008).

Most neurobiological studies of the orthopteran mechanosensory system have been conducted in *Acheta domesticus* (e.g. Edwards and Palka, 1974; Mendenhall and Murphey, 1974; Edwards and Williams, 1981; Bacon and Murphey, 1984; Jacobs et al., 2008; Heußlein et al., 2009), *Gryllus bimaculatus* (e.g. Tauber and Camhi, 1995; Gras and Hörner, 1992; Kanou et al., 1999) and *Gryllodes sigillatus* (e.g. Matsura and Kanou, 1998; Kanou et al., 2006a,b; Takuwa et al., 2008). Many studies under controlled laboratory conditions have revealed different escape strategies among these cricket species. The biological implications of these findings, however, are difficult to establish, given the complete lack of knowledge on the ecology of those species (Dangles et al., 2006a). Conversely, the mechanosensory system of wood-cricket *Nemobius sylvestris* has been the subject of detailed ecological, behavioural and electrophysiological studies, both in the laboratory and in the field (e.g., Coolen et al., 2005; Dangles et al., 2005, 2006a,b,c, 2007, 2008; Steinmann et al., 2006; Dupuy, 2009). The amount of infor-

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doi:10.1016/j.tice.2010.12.001

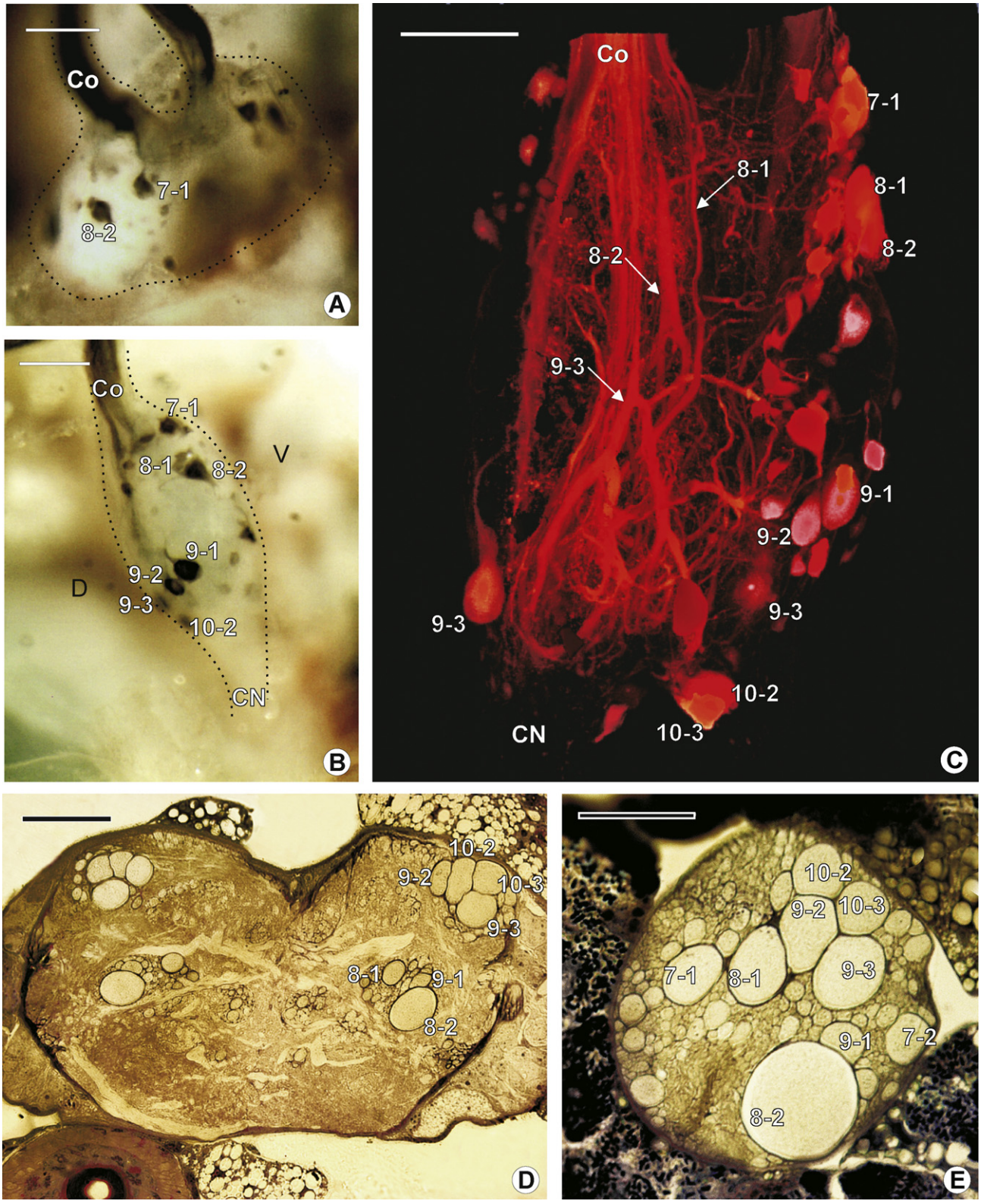

Fig. 1. (A and B) Photographs of the terminal abdominal ganglion *in situ* (as seen in a preparation for electrophysiological recordings) after cobalt backfilling from the thoracic connectives. The position of the soma and the neurites of the giant interneurons are shown. A, anterior view. B, lateral, view. (C) Confocal micrograph of the projection of giant interneurons within the terminal abdominal ganglion labeled by applying rhodamine dextran into the connectives. Co, connective; CN, cercal nerve. (D and E) Micrographs of the transverse sections of the anterior region of the terminal abdominal ganglion and one connective of the ventral nerve cord close to the terminal abdominal ganglion, respectively. The micrographs D and E have been taken from Insausti et al. (2008) to show the identified axons of the giant interneurons. The analyses of histological cuts of labeled interneurons allow us to identify their position in the connectives. The cells 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. Scale bars: A, B, and C, 100 μm; D and E, 50 μm.

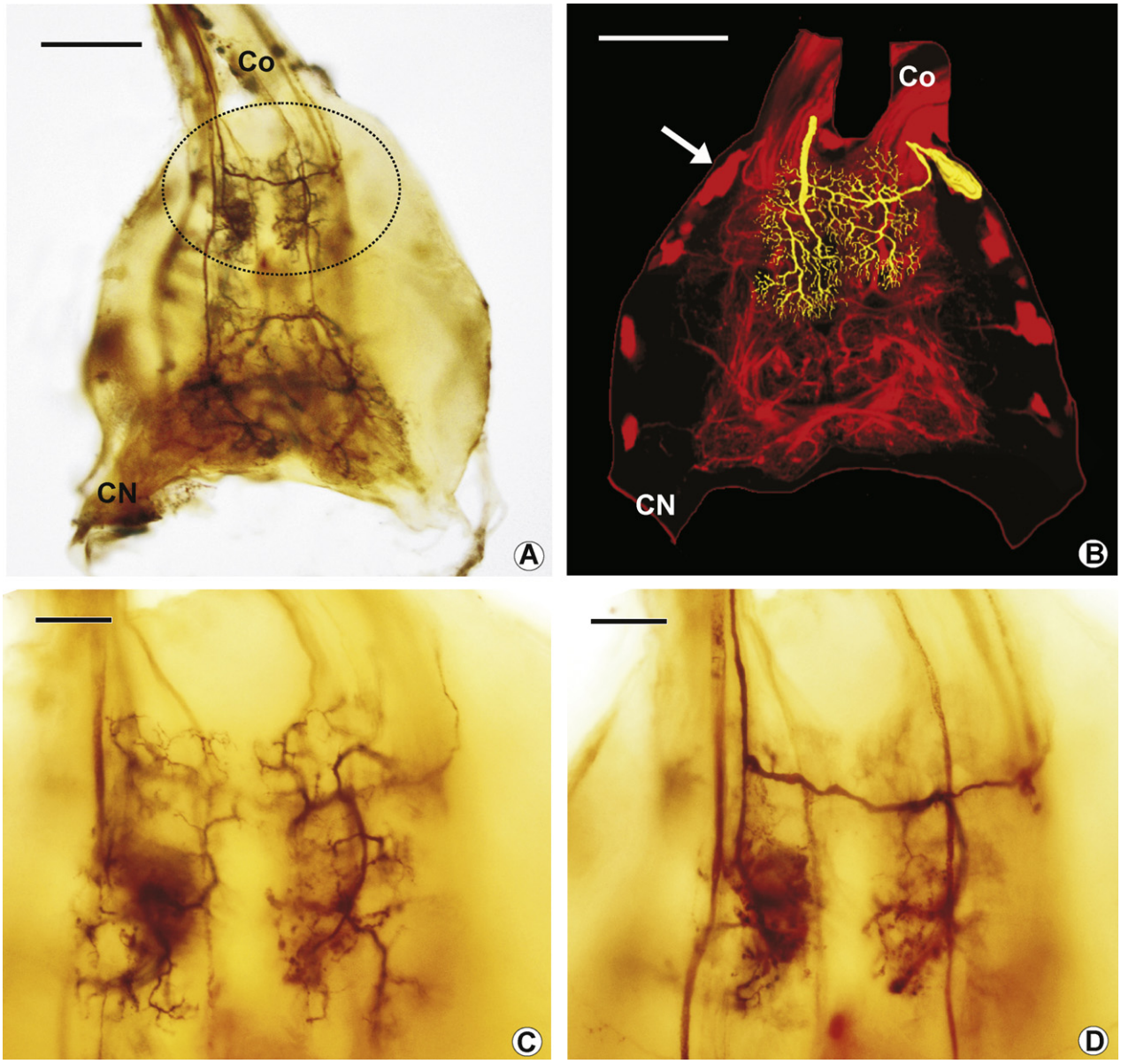

Fig. 2. Photomicrographs of wholemount preparations of retrograde fills of the terminal abdominal ganglion with dye through the connectives. (A) The giant interneuron 7-1a (inside the dotted circle) is revealed by cobalt chloride backfill. (B) Confocal image and three-dimensional reconstruction of the labeled 7-1a giant interneuron within the terminal abdominal ganglion. The arrow shows the soma of the homologous contralateral neuron. (C and D) Details of the arborisations of the giant interneuron 7-1a revealed by cobalt chloride backfill. Co, connective; CN, cercal nerve. Scale bars: A and B, 100 μm; C and D, 30 μm.

mation about its neurobiology is however scant (Insausti et al., 2008) and we do not know to what extent the neural organization of the mechanosensory cercal system is conserved across crickets. So, generalisations are not possible for the neuroanatomical substrate responsible for the functional properties of the system (Dupuy, 2009).

The present work attempts to add a crucial piece of knowledge on the sensory system of *N. sylvestris*, providing detailed anatomical data on the organization of the terminal abdominal ganglion. In our previous study on the terminal abdominal ganglion, we have identified seven pairs of giant interneurons, organized symmetrically (Insausti et al., 2008). In the present study, we characterized the giant interneurons morphology, their fine structure, their dendritic fields and their relationship in the terminal abdominal ganglion neuropiles.

2. Materials and methods

2.1. Animals

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

2.2. Interneuron staining

The giant ascending interneurons were stained with cobalt chloride by retrograde axonal diffusion. The ventral cuticle of the thoracic segments was stripped away, exposing the ventral cord. One or both connectives anterior to the first thoracic ganglion were transected and a Vaseline well was built around it, which

isolated the ganglion from the rest of the 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 1–5 h or overnight at 10 °C in a humid chamber. The terminal ganglion was thereafter dissected out and the cobalt precipitated by treating the ganglion with a freshly prepared solution of ammonium sulphide 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. The cobalt-labeled tissue was either cleared with methyl salicylate for wholemount viewing, or embedded in Durcupan ACM (Fluka-Sigma) and cut horizontally at 30–35 μm.

Photographs and reconstruction drawings were done with the aid of a camera lucida from wholemounts and sections. The digitized images were processed and – when required – modified to enhance contrast with Adobe Photoshop® and Corel Draw® software on a PC.

The same kind of retrograde staining has been performed using a fluorescent dye. A 5% dextran tetramethylrhodamine solution (Molecular Probes, D3308) was applied in the connectives using the same procedure as described for cobalt labeling. The dissected ganglion was fixed overnight at room temperature in 4% paraformaldehyde in Milloning phosphate buffer at pH 7.2. 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 Ar 488 nm and 514 nm, and HeNe 544 nm and 633 nm lasers. Stacks of optical sections were analyzed using ImageJ® and Amira 5® software. Labeled neurons and neuronal arborisations were reconstructed three-dimensionally with Amira 5® software. Neurons were labeled on subsequent pictures of an image stack from the confocal microscope. From this, three-dimensional reconstructions were generated to create a three-dimensional model of neurons. For a better representation of spatial relationships, the reconstructed neurons were integrated to an image of the whole ganglion corresponding to the plane of the focal soma or axon. Branches, however, are not limited to that plane, but project into areas above and below it. It is also worth mentioning, that the ganglion images correspond to a selected plane and preparation, but reconstructions were made from many preparations with different dyes and injection times and across different focal planes. The different times of dye injection allowed getting preparations with only one or a few labeled neurons, these preparations were used for reconstructions. The analysis of labeled neurons from histological cuts allowed us to identify the position of their axons in the connectives.

2.3. Transmission electron and light microscopy

The cricket nervous system was treated following the technique described by Ribi (1987). Briefly, a cricket was fixed for 3 h in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate buffer (pH 7.3) with glucose 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 ACM. Blocks were serially sectioned at 1.5–5 μm using glass knives mounted in a microtome. The sections were stained on a hot plate with Toluidine Blue-Basic Fuchsin and mounted with DPX. For electron microscopy, ultrathin sections were cut with an ultramicrotome using a diamond knife. The sections were doubly stained by uranyl acetate and lead citrate and observed using a JEOL 1010 transmission electron microscope.

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

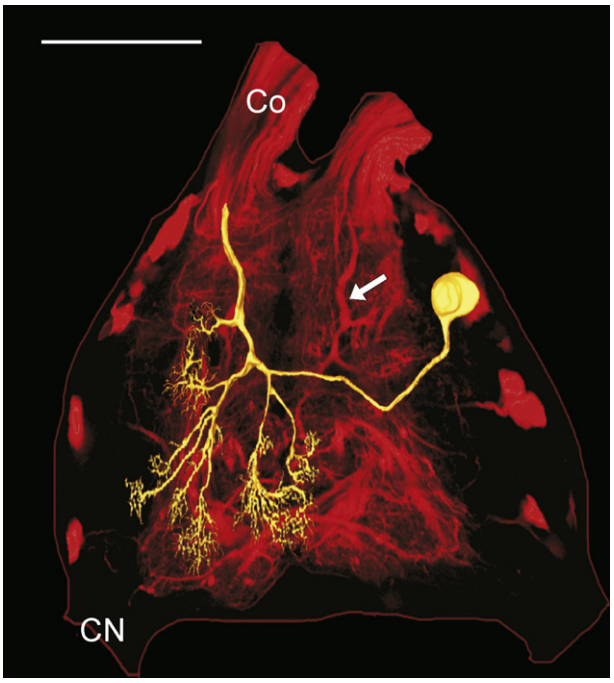

Fig. 3. Confocal image of a wholemount preparation by filled the giant ascending interneurons with fluorescent dye. The three-dimensional reconstruction of the arborisations of the giant interneuron 8-1a is superimposed. The arrow shows the ascending axon of the homologous contralateral neuron. Co, connective; CN, cercal nerve. Scale bar: 100 μm.

3. Results

In the following, we describe in detail: (i) the morphology of the 8 biggest ascending interneurons, called giant interneurons and (ii) their relative position into the terminal abdominal ganglion and within connectives. Subsequently, we analyze the fine structure of the ganglion sheath and giant interneurons. Finally we describe the synaptic circuitry in the cercal neuropile.

3.1. The morphology of the giant interneurons

3.1.1. Giant interneuron 7-1a

The soma of the giant interneuron 7-1a lies in the ventral surface of the terminal abdominal ganglion under the nerve 7 ventral (Fig. 1A–C). Its shape is elongated, of about 55 μm × 25 μm (Figs. 1A and 2B).

This giant interneuron possesses an extensive dendritic field, mostly in the anterior half of the terminal abdominal ganglion and projects on both sides of the ganglion (Fig. 2A and B). The neurite runs slightly anteriorly and then turns posteriorly and dorsally. The cell body fibre crosses the ganglion through the most anterior dorsal commissure. This crossing fibre gives off two ipsilateral branches, and another contralateral to the soma. One of the ipsilateral branches projects anteriorly and arborises ventrally, and the other branch projects posteriorly with dorsal and ventral arborisations. The contralateral branch projects mainly posteriorly and arborises ventrally. Some branches also extend anteriorly (Fig. 2C and D). The axon runs in the ventral intermediate tract (Fig. 1D). It extends anteriorly to enter the connectives (Fig. 1E) and posteriorly to arborise in the anterior quadrant of the terminal abdominal ganglion, contralateral to the soma (Fig. 2B and D). The arborisations of giant interneuron 7-1 superpose with its contralateral homolo-

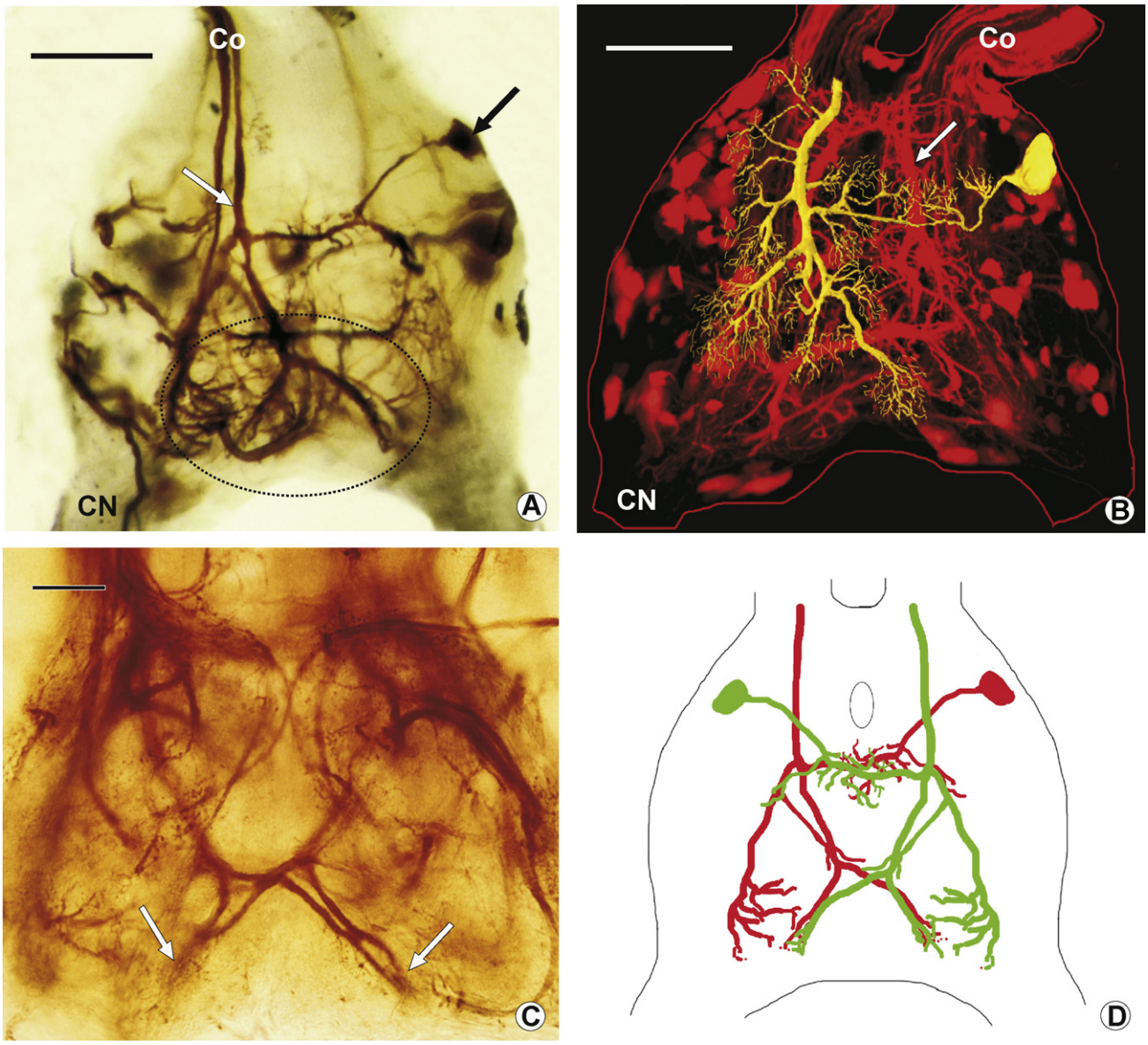

Fig. 4. Wholemount preparations of the terminal abdominal ganglion labeled by retrograde fill with dyes through the connectives. (A) The giant interneuron 8-2a labeled by cobalt chloride backfill. The black arrow shows the cell body and the white arrow the ascending axon. The posterior arborisation is shown inside the dotted circle. (B) Confocal image and three-dimensional reconstruction of the projection of the giant interneuron 8-2a. The arrow shows the ascending axon of the homologous contralateral neuron. (C) Detail of the arborisations of the giant interneuron 8-2a revealed by cobalt chloride backfill. Note the overlapping of the arborisations of the homologous contralateral neurons (arrows). (D) Schematic representation of the terminal abdominal ganglion and the relative positions of the contralateral homologous 8-2a giant interneurons. Co, connective; CN, cercal nerve. Scale bars: A and B, 100 μm; C, 30 μm.

gous interneuron, both covering the entire anterior region of the ganglion.

3.1.2. Giant interneuron 8-1a

The soma of the giant interneuron 8-1a is approximately 40 μm in diameter and lies laterally, dorsal to the horizontal midline of the terminal abdominal ganglion, located at the level of the nerve 8 dorsal (Fig. 1B and C). The neurite rises ventrally from the soma and crosses the ganglion in the anterior commissure of the 8th neuromere to form the axon. The diameter of the axon reaches 14 μm in the middle of the ganglion, and extends posteriorly and ventrally. The axon narrows in the region of the cercal glomerulus, and gives off four main branches which project ventrally and arborise profusely contralateral to the cell body (Fig. 3). The branch projecting to the midline of the ganglion arborises in intimate association with those of giant interneuron 9-3a. The axon fibre joins the ventral intermediate tract in the midline of the terminal abdominal ganglion and leaves it dorsally to the giant interneuron 8-2a (Fig. 1D and E).

3.1.3. Giant interneuron 8-2a

The soma of the giant interneuron 8-2a has a diameter of about 50 μm and is located ventral to the horizontal midline of the terminal abdominal ganglion, under the nerve 8 dorsal and under the body of the giant interneuron 8-1a (Figs. 1A, B, and 4A). The neurite projects ventrally and posteriorly, crossing the ganglion in the anterior commissure of the 8th neuromere together with the neurite of giant interneuron 8-1a. The body fibre arborises repeatedly within the commissure (Fig. 4B). The axon fibre is the largest of the giant interneurons, about 20 μm in diameter, and runs anteri-

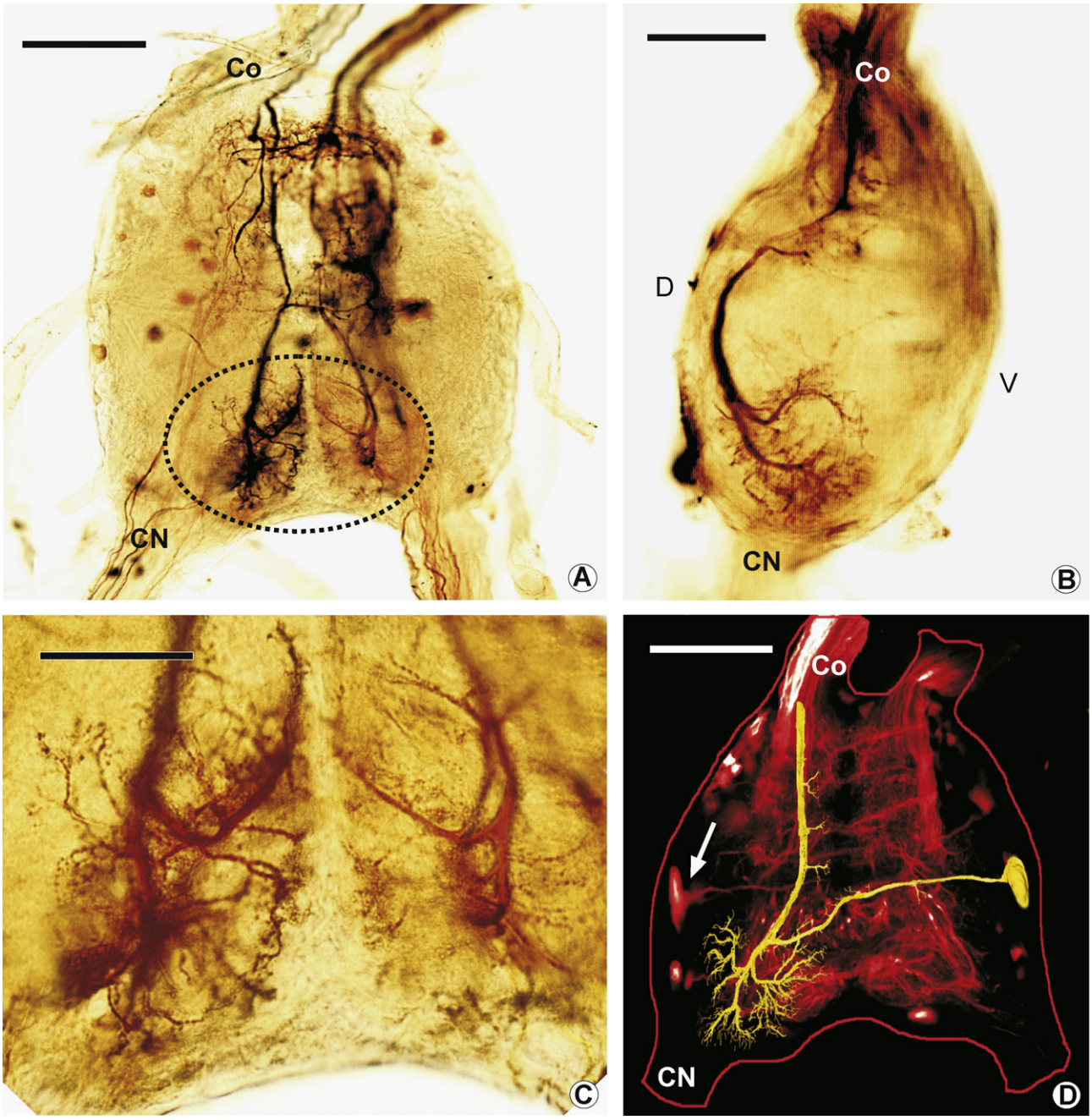

Fig. 5. Wholemount preparations of the terminal abdominal ganglion labeled by retrograde fill with dyes through the connectives. (A and B) Dorsal (A) and lateral (B) view of the terminal abdominal ganglion showing the morphology of the giant interneuron 9-1b, revealed by cobalt chloride backfill (micrographs from Insausti et al., 2008). (C) Detail of the posterior arborisation of the giant interneuron 9-1b (dotted circle in A). (D) Confocal image and three-dimensional reconstruction of the labeled 9-1b giant interneuron within terminal abdominal ganglion. The arrow shows the cell body and neurite of the homologous contralateral cell. Co, connective; CN, cercal nerve; D, dorsal; V, ventral. Scale bars: A, B, and D, 100 μm; C, 50 μm.

orly in the ventral intermediate tract, in close association with the axon of the giant interneuron 9-1b (Fig. 1D). It branches posteriorly in the cercal glomerulus. The anterior segment of the axon fibre gives off several dorsolateral arborisations in the anterior region of the ganglion. Posteriorly, the axon projects ventrally and gives off four main branches. Three of them arborise extensively in the cercal glomerulus contralateral to the soma (Fig. 4A and B). The most lateral branch arborises ventrally in intimate association with the arborisation of the giant interneuron 9-1b. The fourth branch projects to the midline of the terminal abdominal ganglion and to the ipsilateral cercal glomerulus where it arborises in close association with the contralateral homologous 8-2a giant interneuron (Fig. 4C and D).

3.1.4. Giant interneuron 9-1b

The soma of the giant interneuron 9-1b is about 40 μm in diameter and lies ventral to the nerve 9 dorsal (Fig. 1B and C). The neurite projects dorsally, crossing to the contralateral side of the terminal abdominal ganglion in the most anterior commissure of the 9th neuromere. The neurite forms small dendritic branches (Fig. 5A and D). The axon fibre is approximately 12 μm in diameter. The axon projects anteriorly in the ventral intermediate tract,

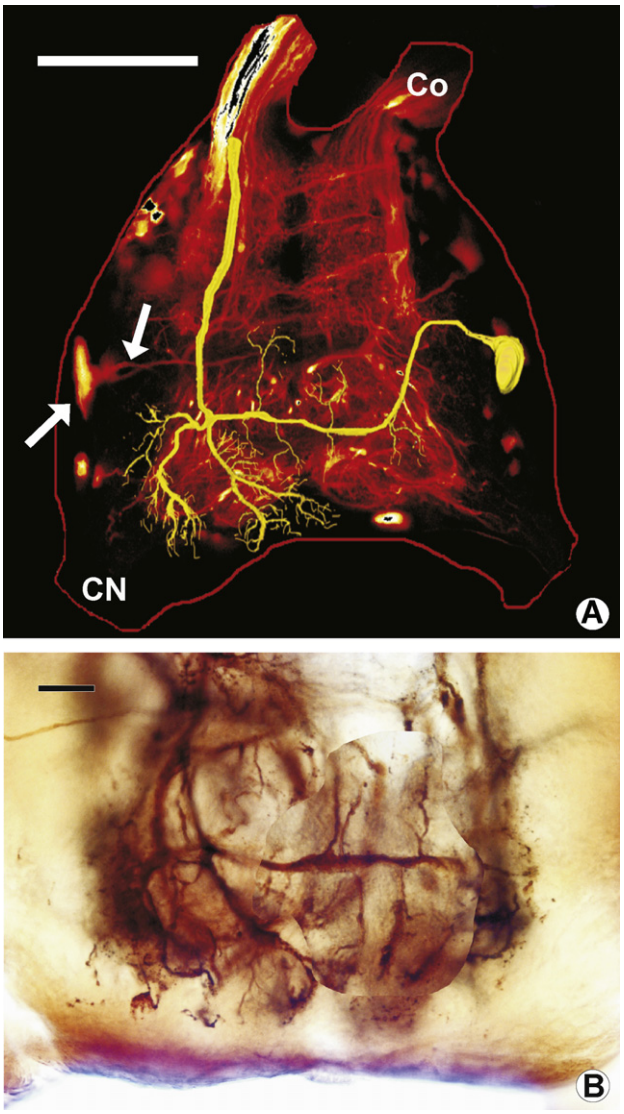

Fig. 6. (A) Confocal image of the labeled 9-2b giant interneuron within terminal abdominal ganglion. The three-dimensional reconstruction of the cell morphology is superimposed. The arrows show the cell body and neurite. (B) Detail of the posterior arborisations and neurite as revealed by cobalt chloride backfill. The image was composed by two images taken at different depths. Co, connective; CN, cercal nerve. Scale bars: A, 100 μm; B, 30 μm.

intimately associated with the axon of the giant interneuron 8-2a (Fig. 1D), and gives off several arborisations. Posteriorly, the axon projects ventrally with two main branches, which arborise profusely in the inferior and medial region of the cercal glomerulus contralateral to the soma, intermingled with the arborisations of the giant interneuron 8-2a (Fig. 5B–D).

3.1.5. Giant interneuron 9-2b

The soma of the giant interneuron 9-2b is approximately 45 μm in diameter and is located in the 9th neuromere, dorsal to nerve 9 dorsal, in the same line with the soma of giant interneuron 9-1b (Figs. 1B, C, and 6). The neurite rises dorsally from the soma, cross over the body fibre of giant interneuron 9-1b and then turns ventrally to cross the midline of the ganglion through the posterior commissure of the 9th neuromere, where it gives off to several slender branches. The axon runs anteriorly in the lateral dorsal tract (Fig. 1D and E), and reaches 15 μm in diameter. Posteriorly, the axon arborises in the cercal glomerulus contralateral to the soma (Fig. 6). Inside the cercal neuropile, the axon emits two main branches, one projects dorsally and the other ventrally.

3.1.6. Giant interneuron 9-3a

The soma of the giant interneuron 9-3a lies posterior-dorsally in the terminal abdominal ganglion, medially in the 9th neuromere (Fig. 1B and C). It is about 45 μm in diameter and the neurite runs across the ganglion medially in the commissure of the 9th neuromere (Fig. 7A and B). The commissural fibre leaves the commissure to form the axon in the midline of the terminal abdominal ganglion. The axon fibre projects anteriorly and joins the lateral dorsal tract (Fig. 1D and E). A fine branch arborises medially in the anterior region of the ganglion. Posteriorly, the axon gives off two main branches. The medial one projects ventrally and arborises in the midline of the ganglion, where it overlaps with the arborisation of the giant interneuron 8-1 and the homologous contralateral (Fig. 7A and C). The external branch projects dorsally and arborises in the basal area of the cercal glomerulus, near the base of the cercal nerve, where the giant interneuron 10-3a also arborises (Fig. 7B).

3.1.7. Giant interneuron 10-2a

The soma of the giant interneuron 10-2a is approximately 50 μm in diameter and is located on the dorsal region near the midline, at the posterior side of the ganglion (Fig. 8A and C). The neurite of this cell rises dorsally from the soma and broadens to cross the ganglion into the posterior commissure of the 10th neuromere (Fig. 8A and C). On the contralateral side, the neuron arborises profusely in the dorsal zone of the cercal glomerulus. The axon projects anteriorly, into the lateral dorsal tract. In the anterior region of the ganglion, a small collateral runs medially from the axon, which reaches about 15 μm in diameter (Fig. 1D).

3.1.8. Giant interneuron 10-3a

The soma of the giant interneuron 10-3a is about 50 μm in diameter and is located, as the soma of giant interneuron 10-2a, on the dorsal region of the posterior side of the ganglion, near the midline (Fig. 8B and C). The neurite projects anteriorly to the contralateral side of the ganglion to form an axon with two branches. One branch crosses the midline of the ganglion into the posterior commissure of the 10th neuromere and arborises posteriorly, in the cercal glomerulus ipsilateral to the body cell (Fig. 8B). The second branch runs anteriorly into the lateral dorsal tract and exits the ganglion in the connective contralateral to the cell body (Fig. 1D and E). This axon fibre projects collaterals, which arborise into the neuropiles of the 9th neuromere contralateral to the cell body. It also projects a slender medial collateral in the neuropile of the 7th neuromere (Fig. 8B). In the anterior region of the lateral dorsal tract, the axon reaches a diameter of about 15 μm.

3.2. The fine structure of the terminal abdominal ganglion

3.2.1. The neural sheath

The entire terminal abdominal ganglion of the wood cricket is surrounded by a fat-body sheath (Fig. 9A). Within this, the neural sheath consists, typically, of the outer acellular neural lamella and the subjacent cellular perineurial layer (Fig. 9B). The neurilemma is about 1–1.5 μm thick and the perineurium approximately 8–10 μm. The ovoid nuclei of perineurial cells are about 9.5 μm long and have single large nucleoli. We observed large amounts of round mitochondria, lipid-like inclusions, glycogen rosettes, greatly developed rough endoplasmic reticulum and free ribosomes in the cytoplasm (Fig. 9C). The cytoplasmic branches of these cells extend between the neuronal cells bodies of the subjacent layer (Fig. 9B).

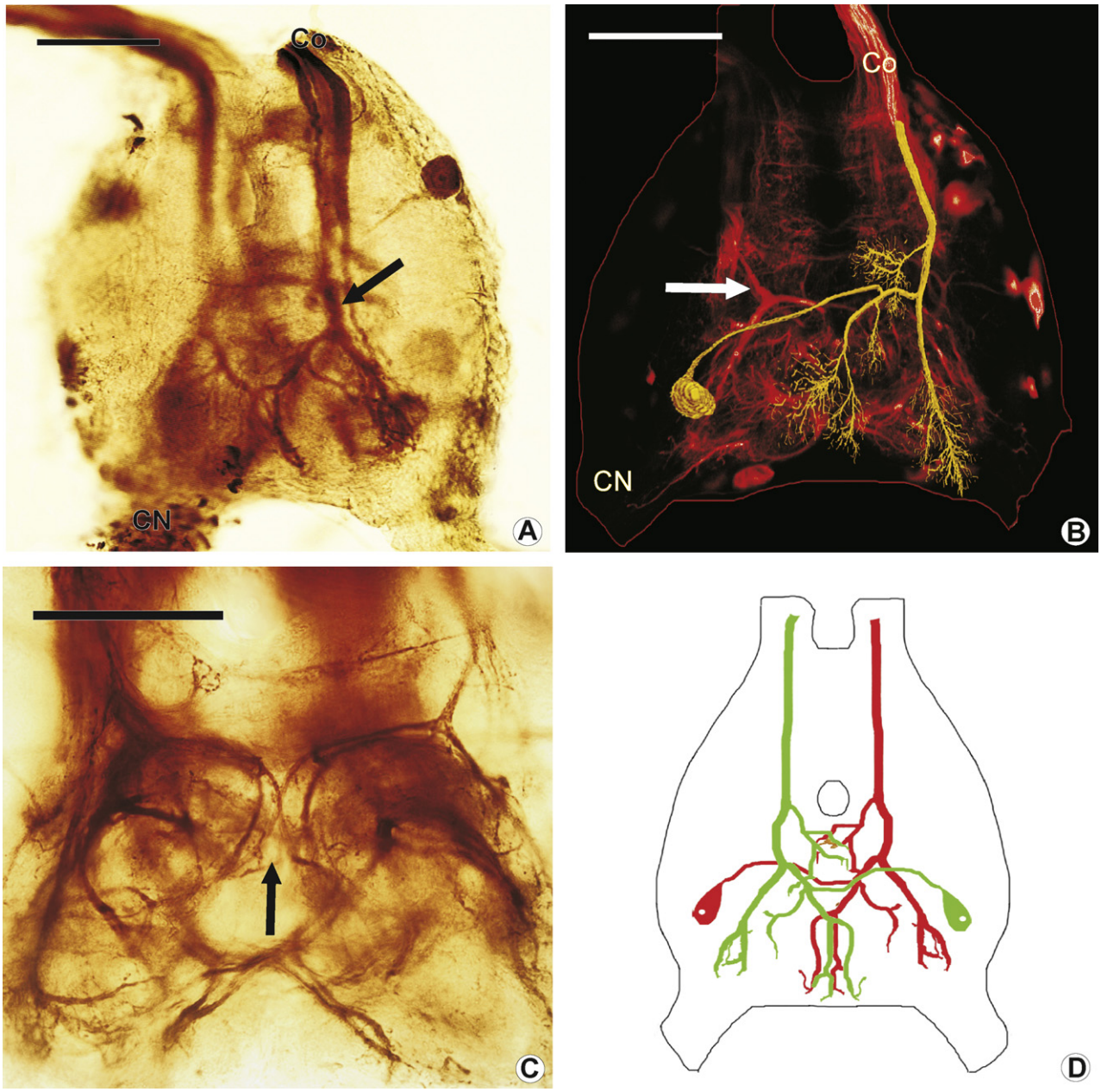

Fig. 7. Wholemount preparations of the terminal abdominal ganglion labeled by retrograde fill with dyes through the connectives. (A) The giant interneuron 9-3a labeled by cobalt chloride backfill. The arrow shows the ascending axon. (B) Confocal image and three-dimensional reconstruction of the labeled 9-3a giant interneuron within terminal abdominal ganglion. The arrow shows the homologous contralateral cell. (C) Detail of the posterior arborisations of the 9-3a giant interneuron labeled with cobalt chloride backfill. The arrow shows the overlapping of the branches of the homologous contralateral neurons. (D) Schematic representation of the terminal abdominal ganglion and the relative positions of the contralateral homologous 9-3a giant interneurons. Co, connective; CN, cercal nerve. Scale bars: A, 100 μm; B, 100 μm; C, 50 μm.

3.2.2. The neuronal cell layer

The neuronal layer is about 24 μm thick and is formed by two or three layers of neurons, depending of the cell size. When a giant interneuron is present, this cell occupies all the width of the layer (Fig. 9A and D). Electrondense glial cells and extracellular spaces and lacunae are present between the neurons (Fig. 9B).

The giant interneurons are monopolar with a large ovoid nucleus and a single big and dense nucleolus. The cytoplasm is extensive, containing numerous free ribosomes, cisternae of rough endoplasmic reticulum and a large amount of mitochondria (Fig. 9D). The giant interneurons contain endocytotic vesicles in the region of contact between the perineurium and the plasma membrane (Fig. 9E). The soma of giant interneurons is surrounded by a thick neuroglia of about 6 μm (Fig. 9D). The neural sheath sometimes exhibits indentations (Fig. 9D and F). The perineural layer is absent and the glial sheath of the giant neuron becomes very thin (about 0.6 μm). The cell surface therefore is very near to the haemolymph in this region. The distance is only 2.5 μm, in contrast to the 10–12 μm in the regular regions (Fig. 9F).

3.2.3. The cercal neuropil

The cercal neuropil occupies the posterior region of the ganglion and contains the larger processes of the giant interneurons, the processes of the local interneurons and the fine fibres of the cercal receptors. The branches of giant interneurons may be distinguished from those of the cercal receptor axons, since they are less electron-dense and contain a smaller number of neurotubules. Highly electron-dense glial cells wrap both types of neurons. The

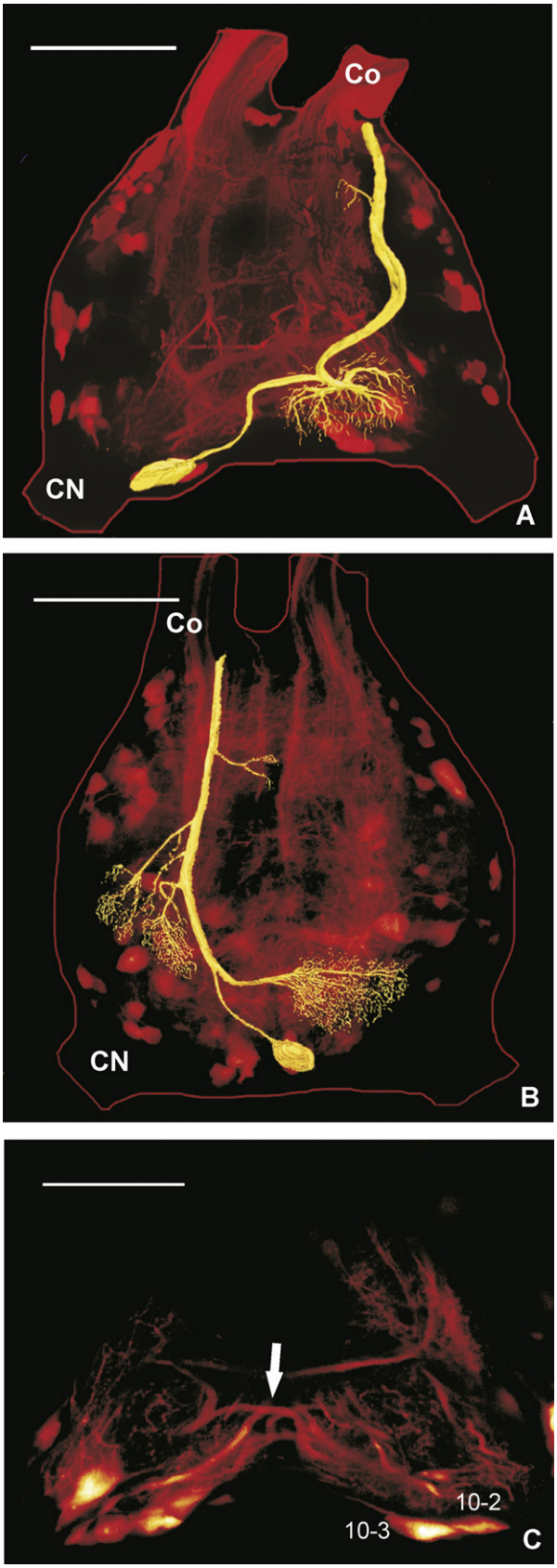

Fig. 8. Confocal micrographs showing the interneurons labeled retrogradely with fluorescent dye. (A and B) Three-dimensional reconstruction of the 10-2a (A) and 10-3a (B) giant interneurons within terminal abdominal ganglion. (C) Detail of the cell bodies of the giant interneurons 10-2a and 10-3a. The arrow shows the crossing of the neurites. Co, connective; CN, cercal nerve. Scale bars: A, 100 μm; B, 100 μm; C, 100 μm.

glial elements may invest a single dendrite process or groups of such dendrites and contain round mitochondria of large size. The processes of the giant interneurons contain large amounts of mitochondria of small size (Fig. 10A).

Synaptic interactions are complex within the cercal neuropile, since all kinds of connections can be found: axo-axonic, dendro-axonic, dendro-dendritic and axo-dendritic contacts (Fig. 10C–F). The presynaptic profiles contain densely packed small synaptic vesicles with electron-dense core. We observed different types of presynaptic structures, including button-like structures (Fig. 10C), dense bar (Fig. 10D and F) and T-shaped bar (Fig. 10E).

In many places the nerve fibres juxtaposed without any intervening glial cell material. These contacts are characterized by a narrowing of the extracellular space between two membranes and sometimes, the apposed axons interdigitated (Fig. 10A arrow, B). We also observed in some regions the presence of synaptic like vesicles near the juxtaposed plasma membranes (Fig. 10A insert).

4. Discussion

4.1. The morphology of the giant interneurons

The giant neurons of *N. 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 occurrence and segmental position of giant interneurons are highly conserved characteristics among the different cricket species (Insausti et al., 2008). A synopsis of the morphology of the giant interneurons of *N. sylvestris* is given in Table 1. The gross structure of the giant interneurons related to wind-reception resembles that of other species of crickets, as has been described elsewhere (Mendenhall and Murphey, 1974; Jacobs and Murphey, 1987; Kanou et al., 2006b). Nevertheless, we have found differences in branching and position of the axons in the ganglion and connectives. In *A. domesticus* the axons of giant interneurons are distributed in three longitudinal tracts (dorso-lateral, ventro-medial and ventro-lateral). In that cricket three neurons were identified in the ventral tracts (7-1, 8-1, 9-1) and four in the dorso-lateral tract (9-2, 9-3, 10-2, 10-3) (Mendenhall and Murphey, 1974). In *N. sylvestris* the giant interneurons clearly occupy two tracts: lateral-dorsal and ventral-intermediate (Insausti et al., 2008). We have identified four axons in the ventral intermediate tract corresponding to the neurons 7-1a, 8-1a, 8-2a and 9-1b, and four in the lateral dorsal tract corresponding to the neurons 9-2b, 9-3a, 10-2a and 10-3a. The axon of the 8-2a interneuron is the largest one, with a diameter of 48 μm measured in the connectives. On the contrary, in *A. domesticus,* Mendenhall and Murphey (1974) described the interneuron 9-1 as the largest; they did not describe the 8-2 interneuron. Jacobs and Murphey (1987) have described the 8-2a interneuron in larvae and adults of *A. domesticus* and embryos of *Gryllus assimilus*, but no reference to the axon size is given, neither to its position in the connective. A partial description of the 8-2a is furthermore available for *G. bimaculatus* (Kohstall-Schnell and Gras, 1994); its branching towards the different neuropiles was however not provided. Edwards and Palka (1974) described in *A. domesticus* the cell that they call “lateral giant interneuron” as the largest axon, which position was indicated as “located superficially in the ventrolateral quadrant”. This could correspond to the ventral intermedial tract of *N. sylvestris*, but given that no illustration was provided, the exact correspondence is therefore hard to establish. Because of its position in the connectives, it is possible to ascribe their “lateral giant interneuron” as equivalent to the 8-2a of wood crickets and their “medial

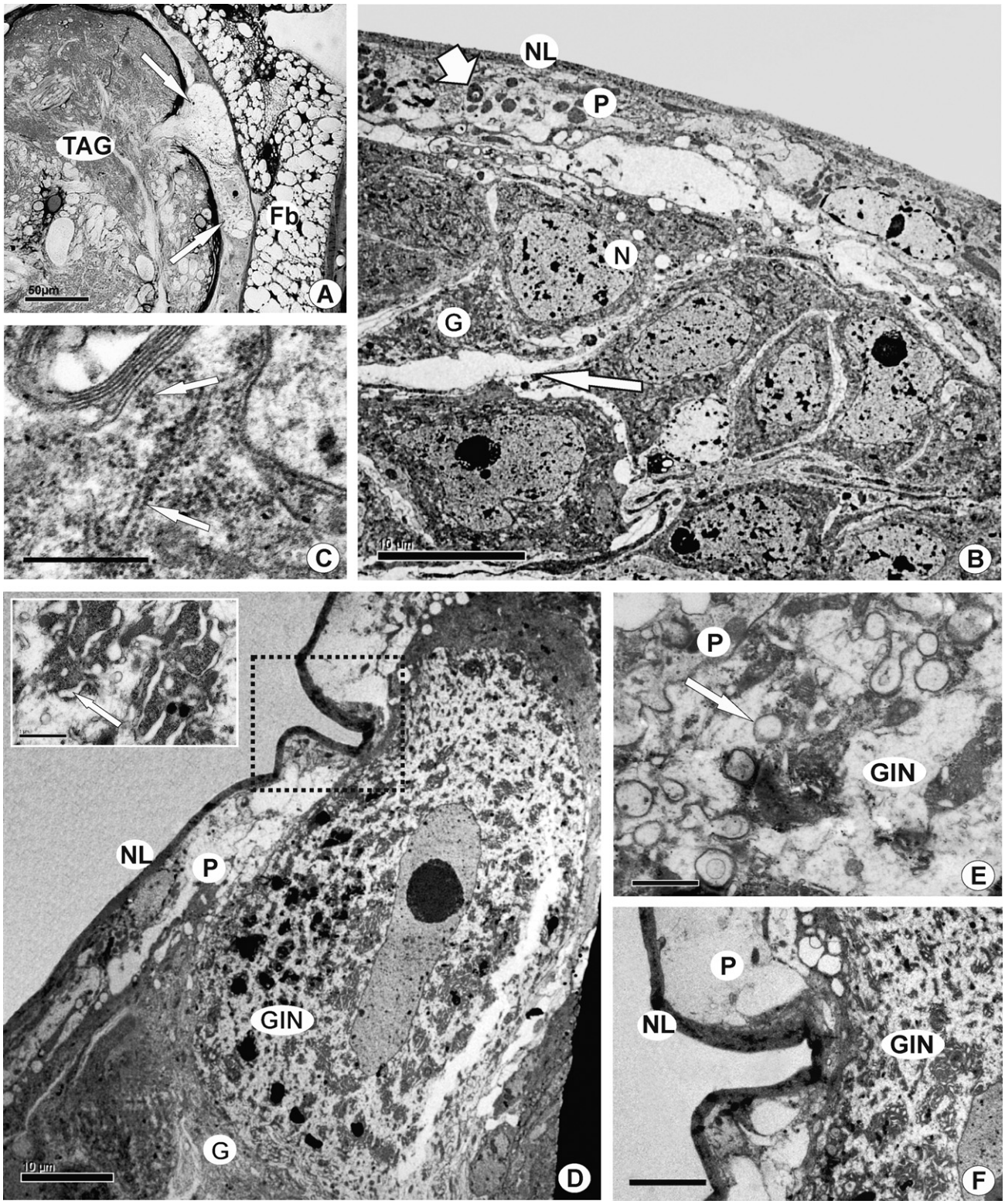

Fig. 9. Photomicrographs of the terminal abdominal ganglion. Light microscopy (A) and transmission electron microscopy (B–E). (A) Transverse section showing the soma of the giant interneurons 8-1a and 8-2a (arrows) within the cortical cell layer. Note the sheath of fat-body surrounded the ganglion. (B) Section through the neural sheath showing the neural lamella, the perineurium and the layer of the neuron cell bodies. The short arrow indicates the round mitochondrias of the perineurial layer. The long arrow shows the extracellular spaces and lacunae between neurons. (C) Detail of the perineurial cell cytoplasm showing the rough endoplasmic reticulum greatly developed and free ribosomes (arrows). (D) A giant interneuron. The cell shows the cytoplasm with the rough endoplasmic reticulum greatly developed (arrow in the insert detail) and large amounts of free ribosomes. The cell body is wrapped with a thick layer of glia. (E) Detail of endocytotic vesicles (arrow) in the region of contact between the perineurium and the plasma membrane of the giant interneurons. (F) Detail of the indentations in the neural sheath (zone of the dotted square in D) where the perineural layer is absent and the glial sheath of the giant neuron very thin. Fb, fat-body; TAG, terminal abdominal ganglion; G, glia; GIN, giant interneuron; N, neuron; NL, neural lamella; P, perineurium. Scale bars: A, 50 μm; B, 10 μm; C, 0.2 μm; D, 10 μm; E, 1 μm; F, 5 μm.

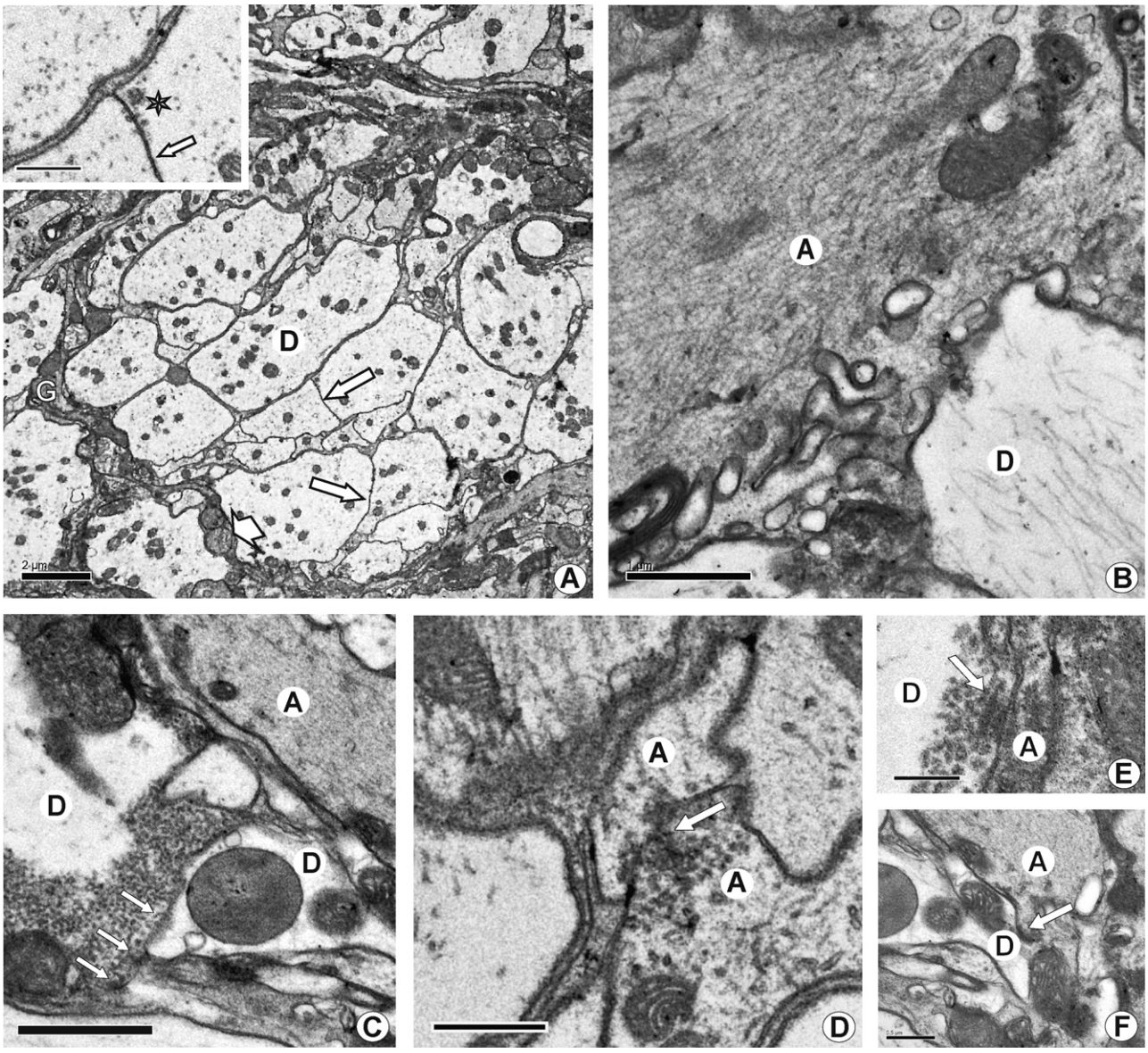

Fig. 10. Transmission electron photomicrographs of the terminal abdominal ganglion. (A) Characteristics of the neural elements in a cross-section through the cercal neuropile. The giant interneurons processes contain large amounts of mitochondria of small size and are bundled by glia (dark cytoplasm). Note the arrangement of large round mitochondria (short arrow) into the glial cytoplasm. The long arrows indicated the "tight junctions" where the dendrites are very close without glia between them (arrow in the insert detail). The asterisk in the insert shows the vesicles of the putative area of chemical synapses. (B) Detail of the region of contact of an axon and a dendrite in close association with the apposed plasmalemma interdigitated. (C–F) Examples of the different synaptic interactions found in the cercal neuropile: dendro-dendritic (C), axo-axonic (D), dendro-axonic (E) and axo-dendritic (F) contacts. The arrows indicate the sites of the presynaptic structures. A, axon; D, dendrite; G, glia. Scale bars: A, 2 µm (insert, 0.5 µm); B, 1 µm; C, 10 µm; D, 0.5 µm; E, 0.25 µm; F, 0.5 µm.

giant interneuron" as equivalent to the 8-1a of *N. sylvestris*. The enounced differences in branching and position may have functional implications, specific for each cricket species. Nevertheless, it is difficult to establish functional correlates, because of the lack of enough information, for some species, ecological (e.g. *Acheta* spp.) or neurophysiological data (e.g. *Nemobius* spp.).

As in *A. domesticus*, the giant interneuron 7-1a of the wood-cricket presents a broad arborisation in the "bristle neuropile", quite away from the cercal glomerulus. This neuron belongs to a group of three neurons of the seventh segment, which receives inputs from the bristle sensilla, and responds to tactile stimuli of the cerci and abdomen (Murphey, 1985). This 7-1a neuron may be triggered when the bristles are touched, leading also to an escape reaction. It is quite frequent that the wind generated by an approaching object does not suffice to evoke the escape of the cricket. In these cases, the touch of the cerci by the object immediately triggers jumping away from the danger (Dangles et al., 2007; Dupuy, 2009). Our study did not include a detailed morphological analysis of the 7-2a interneuron. As this interneuron is not related to wind-detection, nor touching, we only indicate its position in the connective. This neuron was described by Jacobs and Murphey (1987) in *A. domesticus*. It receives inputs from clavate sensilla, which seem to be related to gravity perception (Murphey, 1981; Jacobs and Murphey, 1987).

4.2. The fine structure

The neural sheath of the terminal abdominal ganglion of the wood cricket is essentially similar to those described in other insects (Maddrell and Treherne, 1967; Sohal et al., 1972; Lane, 1974). However, we have found peculiar indentations with absence of perineurium in the regions where a giant neuron is present. In

Table 1
Localization and branching of the giant interneurons (GINs) in the terminal abdominal ganglion (TAG) of the wood cricket *Nemobius sylvestris*.

GIN	Size (μm)	Tract	Neurite commisure	Axon projection	Branches projection
7-1a	Soma: 55 × 25 axon (connective): 25	VIT	7 neuromere anterior-dorsal	Contralat to soma touch neuropile	Extensive dendritic field in the anterior half of the ganglion. The branches projects on both sides
8-1a	Soma: 40 axon (connective): 30	VIT	8 neuromere anterior-ventral	Contralat to soma cercal glomerulus	2 lat-ventral contralat 2 medial-ventral contralat (=9-3a)
8-2a	Soma: 50 axon (connective): 48	VIT	8 neuromere anterior-ventral	Contralat to soma cercal glomerulus	1 ant-dorsal contralat 3 post-contralat 1 post-ipsi (overlapping homologous) → spanning ventrally over the whole glomerulus
9-1b	Soma: 40 axon (connective): 18	VIT	9 neuromere anterior-dorsal	Contralat to soma cercal glomerulus	1 ventral-medial contralat 1 ventral posterior contralat (=8-2)
9-2b	Soma: 45 axon (connective): 30	LDT	9 neuromere posterior-ventral	Contralat to soma cercal glomerulus	1 ventral contralat 2 dorsal contralat
9-3a	Soma: 45 axon (connective): 32	LDT	9 neuromere medial	Contralat to soma cercal glomerulus	1 ventral-medial (=8-1 and overlap homologous) 2 dorsal near cercal nerve (=10-3)
10-2a	Soma: 50 axon (connective): 21	LDT	10 neuromere posterio-dorsal	Contralat to soma cercal glomerulus	Dorsal homogeneous arborisation
10-3a	Soma: 50 axon (connective): 21	LDT	10 neuromere posterior-dorsal	Ipsilat to soma cercal glomerulus	1 contralat to soma, 9 neuromere 1 ipsilat to soma cercal glomerulus dorsal posterior

these spaces, the soma of the giant interneuron is in close proximity to the hemolymph, just separated by the neural lamella and a very thin glial sheath. The neural lamella is not a barrier to electrolyte movement into and out of the central nervous system; its function is only to impart toughness and elasticity (Huddart, 1971). In the region of indentations, the soma of the giant interneuron is very close to the fat-body sheath. In *N. sylvestris*, as in *Carausius morosus*, the fat-body cells form a complete sheath around the ganglion (Maddrell and Treherne, 1966; Lane and Treherne, 1971; Huddart et al., 1973). This fat-body sheath has a trophic role as storage depot for reserve fat, carbohydrates and proteins (Lane, 1974). The indentation in *N. sylvestris* might thus allow a rapid input of nutrients from the fat body towards giant cells.

The second layer of the neural sheath, the perineurium, regulates the metabolic exchange between the central nervous system and the surrounding tissues and body fluids (Huber, 1965). It provides a selectively permeable barrier between the hemolymph and the nervous tissue and plays a role in the maintenance of an optimal ionic milieu of neurons (Lane, 1981). The endocytotic vesicles observed in the region of contact between the perineurium and the plasma membrane of the giant cell are strong indicators of an active exchange of metabolites (Fig. 9E). The wood cricket giant ascend-

ing interneurons are characterized by a large cytoplasmic volume, compared to other ganglionic cells. This is similar to *Calliphora erythrocephala* giant neurons (Strausfeld and Meinertzhagen, 1988), but not to those giant neurons of *C. morosus* (Huddart, 1971). The cells contain numerous free ribosomes, cisternae of rough endoplasmic reticulum and a large amount of mitochondria, characteristics of very active cells. Thus, an active supply of nutrients seems to be needed.

Relatively few data on fine structure of the cricket cercal glomerulus are available, particularly with reference to synaptic relations (Edwards and Palka, 1974). The cercal glomerulus of *N. sylvestris* exhibits the highest possible diversity of synaptic unions (i.e. axo-dendritic, axo-axonic, dendro-axonic and dendro-dendritic), which indicate a complex exchange of information. We have observed that "tight junctions" (as defined by Osborne, 1966; Sotelo and Korn, 1978) are present between nerve fibres. These contacts are characterized by a narrowing of the extracellular space between two membranes, forming a structure similar in appearance to gap junctions. Such structures suggest that electrical synapses may be present in the cercal glomerulus of *N. sylvestris*. Cobalt preparations also gave indications of their presence, provided that when one connective was filled, the "transsynaptic staining" (Strausfeld and Bassemir, 1983) of the contralateral homologue was observed in several giant interneurons. The presence of electrical synapses between the two homologous contralateral giant interneurons suggests that bilateral interactions occur. This probably takes place in the areas of superposition of branching fields (Figs. 4 and 7). Electrical synapses are neuronal gap junctions that mediate fast transmission in many neural circuits. The widespread occurrence of gap junctions has led to the proposal that they play an important part in coordinating cellular signals. Small informational molecules could be directly transmitted between cells via gap junctions. Consequently, this type of communication is an important mechanism for regulating events between cells during embryogenesis and during normal function of organs (Warner et al., 1984; Warner, 1988). It does not exclude the presence of chemical synapses in the same cell. Gap junction channels can contribute to sharpened neuronal activity by synchronizing large neuronal ensembles (Söhl et al., 2005), allowing, for instance, the coordination of the activity of bilateral homologous giant interneurons. Hence, network synchronization results from chemical and electrical synaptic interactions (Friedman and Strowbridge, 2003). The occurrence of mixed synapses allowing chemical and electrical transmission in a single cell are described in mammals and arthropods (Furshpan and Potter, 1959; Strausfeld and Bassemir, 1983; Killmann and Schürmann, 1985; Phelan et al., 1996; Blagburn et al., 1999; Killmann et al., 1999; Söhl et al., 2005). Mixed synapses could also occur in *N. sylvestris*. Indeed, Fig. 10B shows the simultaneous presence of tight contacts between two dendrites, as well as spherical structures resembling synaptic vesicles. It should be noted that, in the only available ultrastructural study of the cricket's terminal abdominal ganglion, Edwards and Palka (1974) reported close contacts between giant fibres collaterals in *A. domesticus*, but no further details as the involved GINs are given. So far as we know, no electrophysiological data on bilateral coupling has been obtained in crickets up to date. Thus, it is difficult to speculate on the functional implications of these contacts, which occurs in particular branches of GINs 8.2a and 9.3a of *N. sylvestris*. Future ultrastructural and electrophysiological research will provide further insight into the functional implications of the existence of electrical synapses in the cercal glomerulus of crickets.

Having now produced the most comprehensive map of giant interneurons in any cricket, we are not only able to confirm results obtained previously, but we are also able to identify all eight giant interneurons within connectives. This asset will be used in upcoming electrophysiological recordings of escaping crickets. The similarities between the organization of the wind-sensitive system of crickets could be seen as reducing the value of conducted detailed comparative studies like the one here presented. Nevertheless, provided that the information concerning each species is limited, we lack of a whole picture even for a single species. It is worth remember that some cricket species constitute classical models in neuroethology, but their ecology is much less known (Dangles et al., 2006a). So, either we invest more effort in studying the biology of *Acheta* or *Gryllus* or the neurobiology of *Nemobius*. We believe that the latter choice would be more straightforward, because of the ease of conducting field experiments involving *Nemobius* compared to the other species (Dupuy, 2009), and our study provides the neuroanatomical basis for this.

Acknowledgment

This work was supported by the European Community (Customized Intelligent Life Inspired Arrays, CILIA project, FP6-IST-016039).

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