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THREE NEURAL GROUPS IN THE FEMORAL CHORDOTONAL ORGAN OF THE CRICKET *GRYLLUS BIMACULATUS*: CENTRAL PROJECTIONS AND SOMA ARRANGEMENT AND DISPLACEMENT DURING JOINT FLEXION

HIROSHI NISHINO[1] AND MASAKI SAKAI[2,*]

[1]*Laboratory of Neuro-Cybernetics, Research Institute for Electronic Science, Hokkaido University, Sapporo 060, Japan* and [2]*Department of Biology, Faculty of Science, Okayama University, Tsushima-Naka-3-1-1, Okayama 700, Japan*

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Summary

The arrangement of neuronal somata and their displacement during joint flexion together with the central projection of the pro- and metathoracic femoral chordotonal organs (FCOs) in the cricket were investigated. The FCO consists of the partially fused ventral and dorsal scoloparia in the proximal femur. The ventrally located neurones (the ventral group) form chain-like rows in which somata became sequentially smaller distally and project their axons ipsilaterally to the dorso-lateral regions, giving off abundant branches and terminating in the region between the dorsal intermediate tract and the ventral intermediate tract in the thoracic hemiganglion. The dorsal scoloparium, composed of small, simply aggregated neurones, projects exclusively to the medioventral association centre (mVAC), which is known to be an auditory neuropile. In addition, another neural cluster (the dorsal group) was found in the proximo-dorsal region of the ventral scoloparium. This was composed of simply aggregated neurones with axons giving off sparse branches dorso-laterally and terminating in the peripheral region inside the mVAC. The somata of these three groups were displaced distally by flexion of the femoro-tibial joint: the ventral group showed the greatest displacement, with the degree of movement depending upon soma location, while the dorsal group and dorsal scoloparium neurones were hardly displaced, possibly because of their strong connection with the cuticle. These properties were similar in both the prothoracic FCO and the metathoracic FCO. Taken together, the above points suggest that there is greater functional differentiation of the FCO than was previously thought.

Key words: cricket, *Gryllus bimaculatus*, femoral chordotonal organ, neural grouping, connective tissues, soma displacement, central projection.

Introduction

The femoral chordotonal organ (FCO) is one of the largest and most complicated proprioceptors in orthopteran insects. The scoloparium, containing a number of sensory neurones, is connected proximally to the femoral cuticle wall by connective tissue and distally to a cuticular apodeme *via* ligaments. The apodeme is attached distally to the tibia, near the joint pivot of the femoro-tibial joint (F-T joint) (Bässler, 1965; Usherwood *et al.* 1968; Burns, 1974).

It has been reported that the FCO consists of two scoloparia that are present in all the legs of the stick insect *Carausius morosus* (Füller and Ernst, 1973) and the tettigoniid *Decticus albifrons* (Theophilidis, 1986), in the pro- and mesothoracic legs of the locust *Schistocerca gregaria* (Burns, 1974) and in the metathoracic leg of the weta (Matheson and Field, 1990) and the cricket *Acheta domesticus* (Nowel *et al.* 1995). In the locust, the neurones in the two scoloparia of the mesothoracic FCO have different central projections in the thoracic ganglion (Field and Pflüger, 1989). Physiologically, one of the scoloparia (in the locust, the distal scoloparium; in the stick insect, the ventral scoloparium) consists of large neurones, responds to slow and large movements of the apodeme and serves as a transducer of F-T joint movement, which mediates postural resistance reflexes (Field and Pflüger, 1989; Kittmann and Schmitz, 1992). The other scoloparium (in the locust, the proximal scoloparium; in the stick insect, the dorsal scoloparium) consists of small neurones, has no detectable reflex in the femoral muscles (Field and Pflüger, 1989; Kittmann and Schmitz, 1992) but responds strongly to vibrations of the apodeme rather than to slow and large movement of the apodeme (Field and Pflüger, 1989). These results indicate that the FCOs in the locust and stick insect are functionally differentiated.

*Author for correspondence (e-mail: masack@cc.okayama-u.ac.jp).

So far, however, most studies of the FCO have been carried out in the locust and stick insect. In the cricket, the ultrastructure of the ligament and apodeme has been studied (Nowel *et al.* 1995), but no information about the neural arrangement and central projection of the FCO is available. In the present study, we have examined these features of the FCO in the cricket *Gryllus bimaculatus* and measured the displacement of FCO neurones during stretching of the apodeme as a basis for a neuroethological study (Nishino and Sakai, 1996).

Materials and methods

Male and female crickets *Gryllus bimaculatus* DeGeer were used 3–15 days after the imaginal moult.

Gross anatomy

The pro- and metathoracic femoral chordotonal organs (FCOs) were examined. The mesothoracic FCO was excluded because it was very similar in structure to the prothoracic FCO. To gain access to the FCO, a small U-shaped incision was made in the exoskeleton (consisting of cuticle and hypodermis) over the scoloparium. The U-shaped portion of the cuticle was carefully stripped off with fine tweezers, and the hypodermis was then skinned off without damaging the surrounding tissue. To examine the mechanical relationship between the FCO and surrounding tissues, preparations were first stained with Methylene Blue, and then either the scoloparium or the connective tissue was pushed and pulled with fine tweezers to estimate the stiffness of the connective tissue.

Backfilling of neurones in the FCO

Animals were cold-anaesthetized on ice–water for 10–15 min and fixed ventral side up on a cork plate. The ventral thorax was opened to give access to the main leg nerve (N5, see Fig. 1). To stain the peripheral innervation of the FCO, N5 was cut at the proximal level of the coxa and its peripheral cut end was placed in the tip (internal diameter, 500 μm) of a polyethylene capillary tube (Hematlon) filled with 0.25 mol l^{-1} nickel–cobalt mixture ($NiCl_2$:$CoCl_2$=22:3) (after Sakai and Yamaguchi, 1983). The preparation was left in a moist chamber at 6 °C for 15–20 h. The leg was then cut off at the proximal end of the coxa and fixed on a cork plate with the anterior side of the femur up. The leg was immersed in cricket saline, containing (in mmol l^{-1}): NaCl, 140; KCl, 9.9; $CaCl_2{\cdot}2H_2O$, 2.2; $MgCl_2{\cdot}6H_2O$, 4.8; glucose, 43; *N*-Tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (Dotite Tes), 2.5, adjusted to pH 7.2 with NaOH, reacted with rubeanic acid and fixed in Carnoy's fixative. It was then dehydrated in an ethanol series and cleared in methyl salicylate. The scoloparium was photographed or sketched using a *camera lucida*. The total number of stained neurones was counted under a microscope by continuously changing the focus. The size of the soma of each neurone was measured using a micrometer and is presented as the mean value for the long and short axes. Mean soma size and the standard deviation (S.D.) were measured in 10–12 preparations.

Central projections of the FCO neurones

Selective forward-filling was performed to examine the central projection of the individual scoloparia (ventral and dorsal scoloparia). However, in *G. bimaculatus*, the ventral scoloparium contained two subgroups of neurones, a ventral and a dorsal group (Zill, 1985), while the dorsal scoloparium neurones were dealt with here as a single group.

In the ventral scoloparium, the ventral and dorsal groups of neurones were embedded in connective tissues so that they were easily separated with an electrolytically tapered tungsten wire. The tissue of the cut end was torn with a tungsten wire to facilitate permeation and then placed into the tip (internal diameter, 50–80 μm) of a glass microelectrode filled with the nickel–cobalt mixture. For the dorsal scoloparium neurones, a similar treatment was used, but the cut end for diffusion was the dorsal ligament containing dendrites. Preparations were left in a moist chamber at 6 °C for 36–48 h. The thoracic ganglion was then reacted with rubeanic acid and fixed in 4 % neutral formaldehyde at 6 °C for 12 h. It was dehydrated and intensified using Timm's method (Bacon and Altman, 1977).

The results were derived from the following successfully stained preparations: (a) prothoracic FCO, eight ventral groups and seven dorsal groups from ventral scoloparia, and five dorsal scoloparia; (b) metathoracic FCO, eight ventral groups and 15 dorsal groups from ventral scoloparia and four dorsal scoloparia. Some of the ganglia from successful preparations were embedded in Paraplast wax, serially sectioned at 18 μm and mounted in Entellan. Sections were counterstained, if necessary, with 0.1 % Toluidine Blue. These preparations were observed under a Nomarski interference microscope and sketched using a *camera lucida*. We referred to Matheson (1992) for the names of axonal branches and to Tyrer and Gregory (1982) and Pflüger *et al.* (1988) for the names of neuropiles and commissures.

Displacement of retrogradely stained FCO neurones

The positional change of FCO neurones caused by apodeme stretch was measured at different F-T joint angles in the prothoracic (*N*=6) and metathoracic (*N*=6) FCOs from 12 animals. The F-T joint was flexed by steps from 160 ° (full extension) to 135 °, 90 °, 45 ° and 0 ° (full flexion) in a minimally dissected preparation (backfilled, reacted with rubeanic acid but not fixed in Carnoy's fixative). The preparation was photographed successively at each F-T joint angle under a binocular microscope (×180). Soma displacement in each neural group was measured on 1–3 representative neurones which were located at particular positions with respect to a reference line. In the ventral group, the most distal, intermediate and proximal neurones were chosen. In the dorsal group and dorsal scoloparium neurones, one neurone, located at nearly the same level as a proximal neurone of the ventral group, was arbitrarily chosen as the representative.

Results

Although back- and forward-filling of distally located small neurones with soma diameters of 7–15 μm by axonal diffusion was difficult in the metathoracic FCO of the locust (Matheson, 1990; Matheson and Field, 1990), we were able to stain even smaller neurones with soma diameters of 4–8 μm provided that the diffusion time was long enough. This was possibly due to the short distance (approximately 6 mm) between the FCO and the metathoracic ganglion in the cricket compared with the longer distance (approximately 20 mm) in the locust.

The anatomy of the proximal region of the femur

Prothoracic leg

The prothoracic FCO consists of the ventral scoloparium and the dorsal scoloparium located in the dorsal, proximal part of the femur (Fig. 1A, dotted). The ventral scoloparium is connected to the ventral ligament, and the dorsal scoloparium to the dorsal ligament. The ventral ligament is stiff while the dorsal ligament is elastic, possibly because the former contains a cuticular core (Nowel *et al.* 1995). Both ligaments are connected into an apodeme which extends almost half the length of the femur. The distal end of the apodeme is attached to the tibia dorsal to the joint pivot.

The main leg nerve, N5, gives rise to nerve 5B1 (N5B1) and nerve 5B2 (N5B2) before entering the femur (after Theophilidis and Burns, 1979). Within the femur, N5B1 gives rise to the motor nerve innervating the extensor tibia muscle, the sensory nerve innervating campaniform sensilla and ventral hair sensilla and the FCO nerve. Both the cuticular nerve innervating hair sensilla on the anterior cuticle and the receptor strand innervated by the strand receptor (Bräunig, 1985) emanate from the FCO scoloparia and run dorsally parallel to the ligaments.

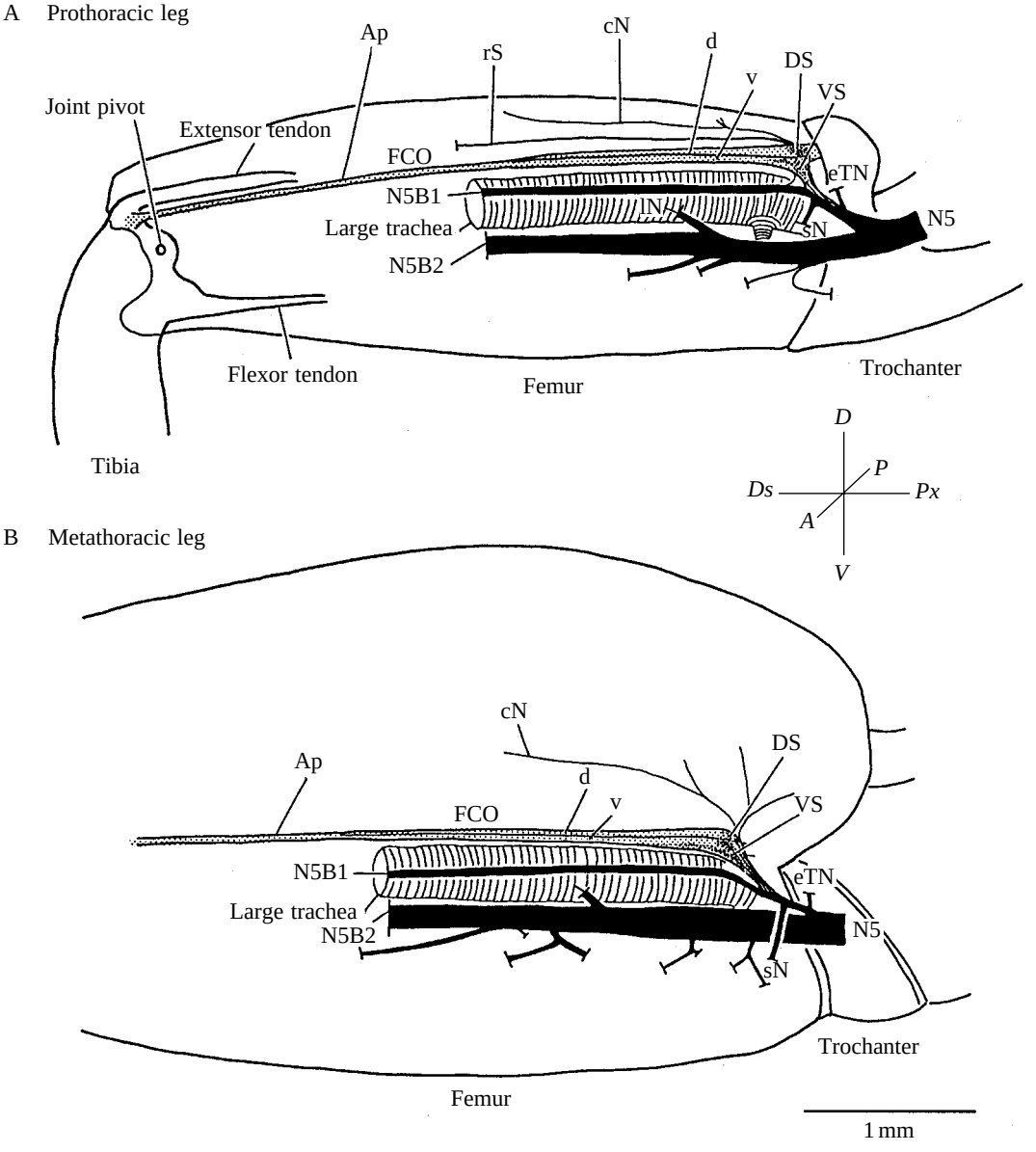

Fig. 1. Anterior view of a preparation back-filled with the nickel–cobalt mixture showing the innervation pattern of the femoral chordotonal organ (FCO) and related nerve roots. Dotted area shows the FCO. (A) Prothoracic leg. (B) Metathoracic leg. Ap, apodeme; cN, cuticular nerve; d, dorsal ligament; DS, dorsal scoloparium; eTN, extensor nerve; lN, lateral nerve; N5, nerve 5; N5B1, nerve 5B1; N5B2, nerve 5B2; rS, receptor strand of the strand receptor; sN, sensory nerve; v, ventral ligament; VS, ventral scoloparium. In this and subsequent figures, abbreviations for directions are as follows: *D*, dorsal; *V*, ventral; *Px*, proximal; *Ds*, distal; *A*, anterior; *P*, posterior; *M*, medial; *L*, lateral.

Metathoracic leg

The metathoracic FCO is located in the most proximal region of the femur just below the antero-medial cuticle, between the extensor and flexor muscles (Fig. 1B). It is composed of the ventral scoloparium and the dorsal scoloparium. These two are connected to an apodeme by the ventral and dorsal ligaments at a point one-third of the way along the femur from its proximal base.

The innervation of the metathoracic FCO is essentially similar to that of the prothoracic FCO. The receptor strand of the strand receptor emanates from the posterior side of the FCO scoloparia (not shown). The large multipolar muscle receptor innervated by a branch of the cuticular nerve in the locust (Matheson and Field, 1995) was not observed in the cricket.

Scoloparia and their sensory neurones in the prothoracic FCO

Fig. 2A shows retrogradely stained neurones in the prothoracic FCO scoloparia with the F-T joint held at 120°. The ventral and dorsal scoloparia, fused in the proximal region, are slightly overlapped, with the ventral scoloparium being anterior (forward) and the dorsal scoloparium posterior

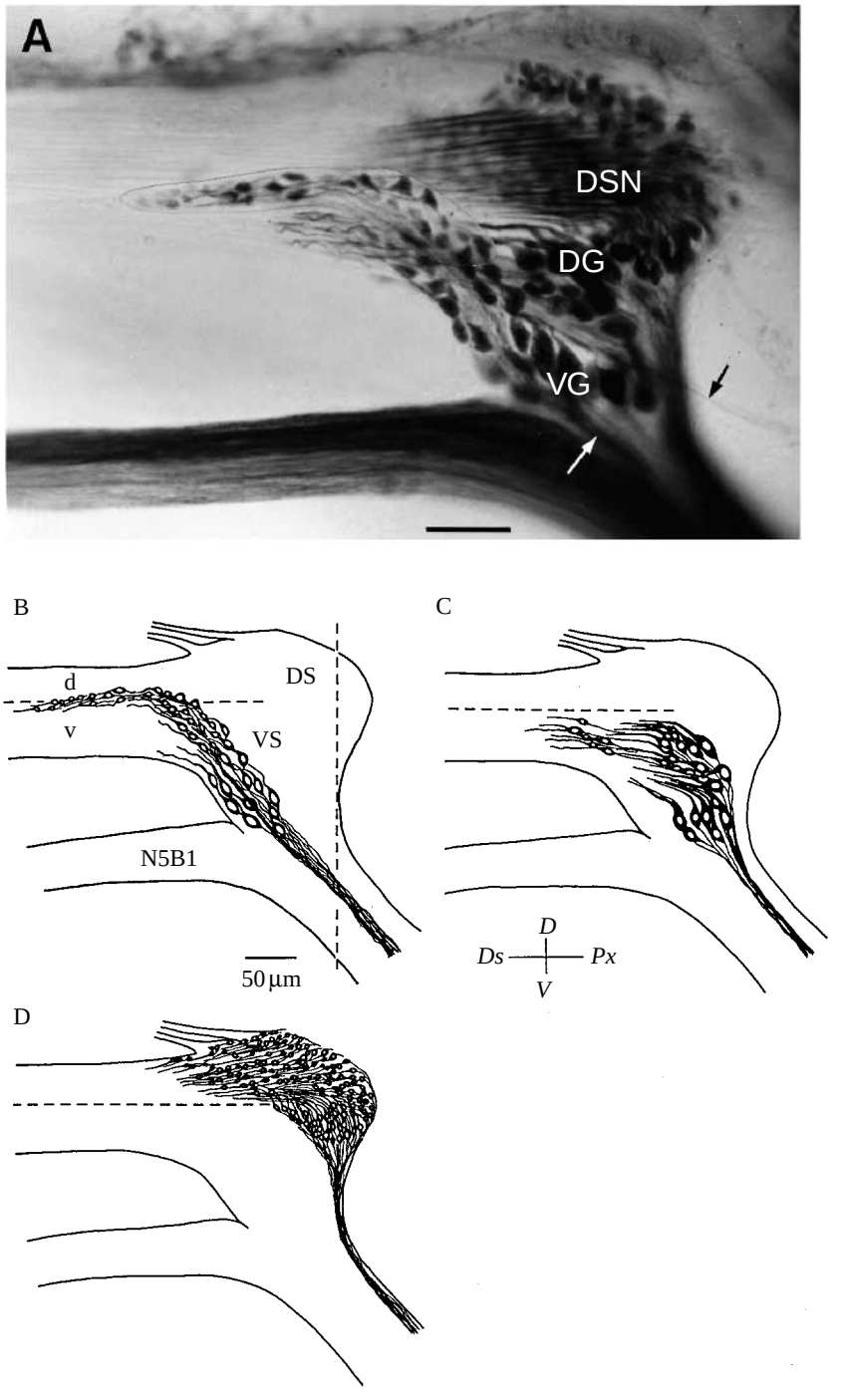

Fig. 2. Three sensory neurone groups in the prothoracic FCO. (A) Photograph of the scoloparia filled with nickel–cobalt mixture. A white arrow indicates the axon bundles innervating the distally located small neurones in the ventral group, a black arrow marks the ligament connecting the scoloparia and the proximal cuticle (l in Fig. 5A). VG, ventral group; DG, dorsal group; DSN, dorsal scoloparium neurones. Scale bar, 50 µm. (B) *Camera lucida* drawing of the ventral group. Soma diameter (6.5–18.5 µm) in this preparation is sequentially smaller towards the distal end. (C) Dorsal group. Neurones (8.5–19.5 µm in diameter) were concentrated proximally in the dorsal region. (D) Dorsal scoloparium neurones. Soma diameters range between 5.5 and 13.3 µm. The border between the ventral scoloparium and the dorsal scoloparium is indicated by a horizontal broken line in B–D. The vertical broken line in B shows the reference line used for the measurement of soma distribution (see Fig. 4). For other abbreviations, see Fig. 1.

(backward). The dendrites of all sensory neurones in the two scoloparia are directed towards the ligaments. The sensory neurones in the ventral scoloparium are divided into two subgroups, a ventral and a dorsal group upon the basis of soma location and the arrangement of somata and dendrites. The axons of the distally located smaller neurones in the ventral group run centrally as a bundle on the ventral side of the FCO nerve (Fig. 2A, white arrow), while those of the proximally located larger neurones run in the intermediate part of the FCO nerve. In contrast, axons of the dorsal group merge with those of the dorsal scoloparium neurones and run out as a nerve bundle on the dorsal side of the FCO nerve. Although the dorsal scoloparium neurones may form several clusters (its ventrally located neurones are somewhat larger than its dorsally located neurones), partitioning of the scoloparium into distinct regions is difficult, and the sensory neurones of the dorsal scoloparium are regarded here as a single group. Fig. 2B–D illustrates *camera lucida* drawings of the two subgroups in the ventral scoloparium and the dorsal scoloparium neurones shown in Fig. 2A.

Ventral scoloparium neurones

Ventral group. The number of stained neurones was 43±3 (mean ± S.D., *N*=8) in a tapering chain-like cluster with fewer neurones in the distal region (Fig. 2B). The somata of these neurones vary in size (11.0±3.2 µm, *N*=10) but are arranged in the organ in order of size, with smaller ones situated distally and larger ones proximally. The distal neurones are embedded in the soft connective tissue attaching them to the dorsal side of the ventral ligament. When the F-T joint is extended to 120 °, and the apodeme is slack, almost all the dendrites appear to be wavy in shape. At smaller angles, they become less wavy, starting from the distal region, and finally become straight at 0 °.

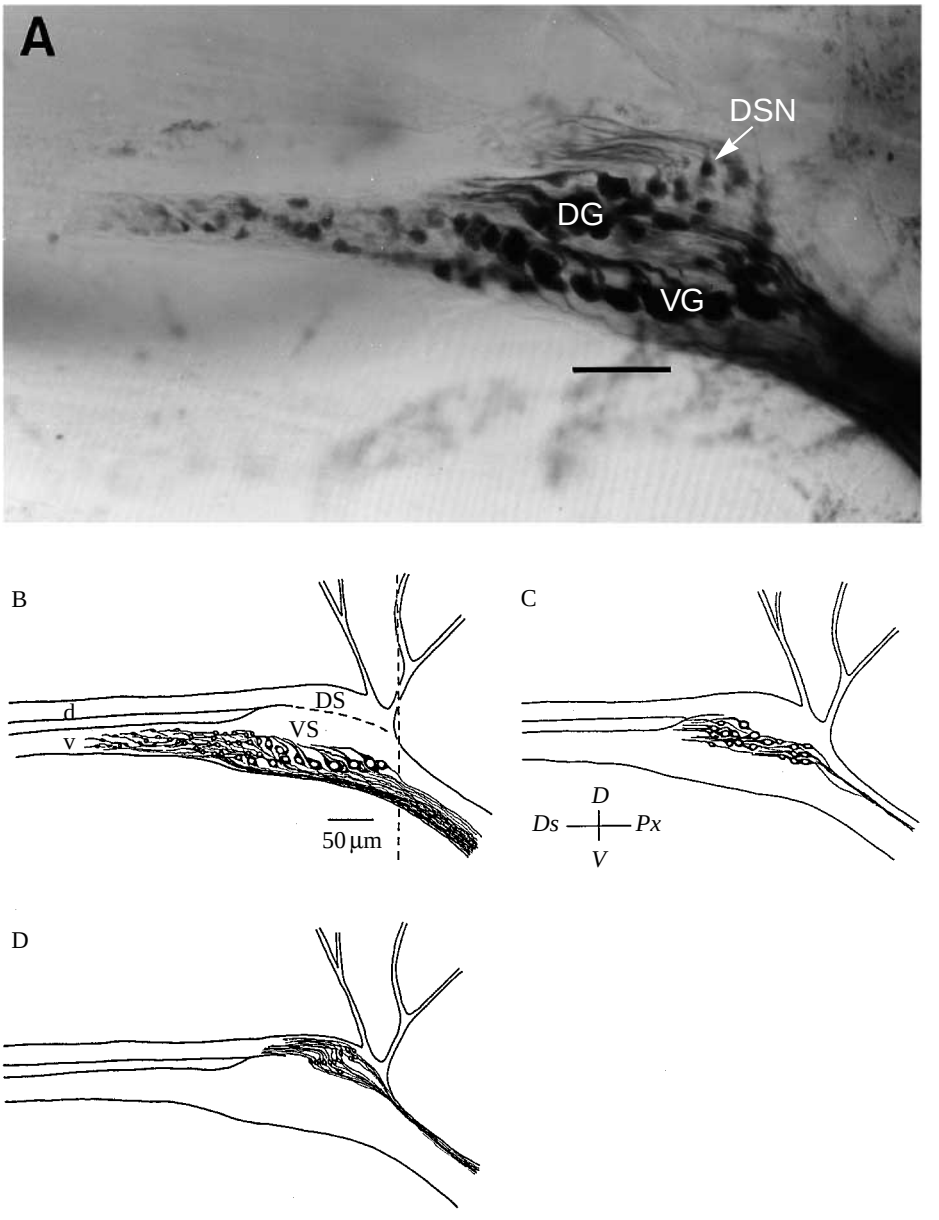

Fig. 3. Three sensory neurone groups in the metathoracic FCO. (A) Photograph of scoloparia filled with nickel–cobalt mixture. VG, ventral group; DG, dorsal group; DSN, dorsal scoloparium neurones. Scale bar, 50 µm. (B) *Camera lucida* drawings of the ventral group. Neurones (5.5–18.5 µm in diameter in this preparation) are sequentially arranged with small somata situated distally. (C) Dorsal group. Neurones (7.9–16.2 µm in diameter) are concentrated proximally in the dorsal region. (D) Dorsal scoloparium neurones (7.2–10.8 µm in diameter) are concentrated in the most dorsal region. The vertical broken line in B shows the reference line for the measurement of soma distribution (see Fig. 4). For other abbreviations, see Fig. 1.

Dorsal group. The number of stained neurones was 25±3 (*N*=8) and these are concentrated in the dorso-proximal region (Fig. 2C). Many of their somata are large (11.7±2.6 μm, *N*=10) with thicker dendrites than those of the ventral group. The dendrites of the proximally located neurones are bent, but the bend varies between individual neurones. The smaller the F-T joint angle, the larger is the bend in the hooked dendrites. No particular soma arrangement was noted in these preparations.

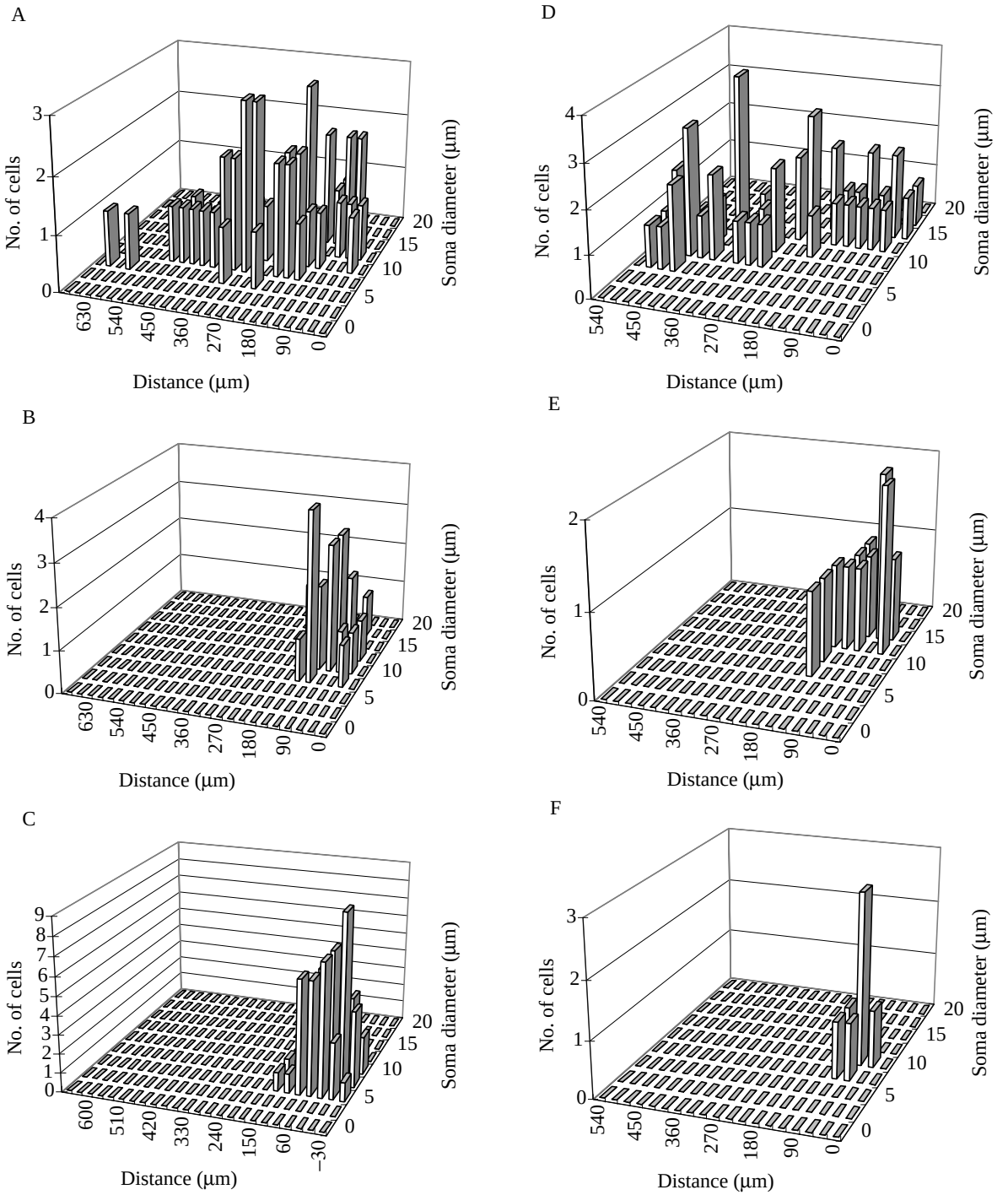

Fig. 4. Three-dimensional plots of the soma size, number and position for the three neural groups in the FCO. The data were obtained from preparations in which the femoro-tibial (F-T) joint was fixed at 0° (full flexion). (A–C) Prothoracic FCO. (A) The ventral group (*N*=48); (B) the dorsal group (*N*=23); (C) the dorsal scoloparium neurones (*N*=68). (D–F) Metathoracic FCO. (D) The ventral group (*N*=48); (E) the dorsal group (*N*=13); (F) the dorsal scoloparium neurones (*N*=7). The reference line (0 μm on the abscissa) corresponds to the vertical broken line in Fig. 2B for A–C and in Fig. 3B for D–F.

Dorsal scoloparium neurones

The dorsal scoloparium contains 110–130 stained neurones in the proximal region of the FCO that show no particular arrangement (Fig. 2D). The somata are small and show a relatively small range of sizes (8.0±1.9 μm, *N*=12).

Scoloparia and their sensory neurones in the metathoracic FCO

Fig. 3A shows a photograph of retrogradely stained neurones of scoloparia in the metathoracic FCO with the F-T joint held at 120°. Neural clustering, dendritic shape and axonal innervation are essentially similar to those in the prothoracic FCO. For the ventral scoloparium, the ventral and dorsal groups are more easily separable than those in the prothoracic FCO because the dorsal group neurones are located apart from the proximal extension of the ventral ligament and are packed into a restricted dorsal region. The dorsal scoloparium is smaller than that in the prothoracic FCO and does not appear to form subgroups of neurones. Fig. 3B–D illustrates *camera lucida* drawings of the three neural groups shown in Fig. 3A.

Ventral scoloparium neurones

Ventral group. There were 51±7 stained neurones (*N*=6) arranged in several chain-like clusters to form more gentle curves compared with that of the prothoracic FCO (Fig. 3B). The somata varied in size (10.2±4.3 μm, *N*=10) and became sequentially smaller distally. Only the distally located neurones were embedded in the soft connective tissue attaching to the anterior side of the ventral ligament.

Dorsal group. The number of stained neurones found was 18±2 (*N*=7) (Fig. 3C). Soma diameter is moderate and not as variable (12.1±2.1 μm, *N*=10) as that of the ventral group neurones. Neurones are concentrated in the dorsal part of the scoloparium and most of their dendrites are bent in their proximal portions.

Dorsal scoloparium neurones

The number of stained neurones was 10±4 (*N*=6), which was much lower than the number in the prothoracic FCO. The neurones have small somata (9.0±1.1 μm, *N*=10) aggregated in the proximal region of the scoloparium (Fig. 3D).

These results are summarized in three-dimensional plots (Fig. 4) in which the position of each soma is plotted with reference to the vertical dashed line in Figs 2B, 3B. The F-T joint angle was fixed at 0°. In the prothoracic FCO, neurones in the ventral group are arranged from larger to smaller distally (Fig. 4A), while no such relationship is present in the dorsal group (Fig. 4B) or the dorsal scoloparium neurones (Fig. 4C). The latter two resemble each other in soma location and spatial distribution. A similar tendency was observed in the metathoracic FCO (Fig. 4D–F).

Connections of the FCO with surrounding tissues

While the apodeme was free in the femur except for its distal connection with the joint pivot, the scoloparia were connected to the surrounding structures. To estimate the possible sources other than the apodeme which could activate FCO neurones, the nature of the connection between the scoloparia and the surrounding structures was investigated.

Fig. 5A shows semi-schematic drawings of the connective tissue binding the two scoloparia with the trachea, nerve bundles and cuticular wall in the prothoracic FCO. The connection with the trachea consisted of elastic connective tissue found in the fork (lightly shaded region indicated by an asterisk, Fig. 5A) between the FCO and N5B1. N5B1 is firmly attached to the large trachea longitudinally by connective tissue (not shown) and, thus, the scoloparia could be affected by tracheal movement. Both elastic and stiff connective tissues

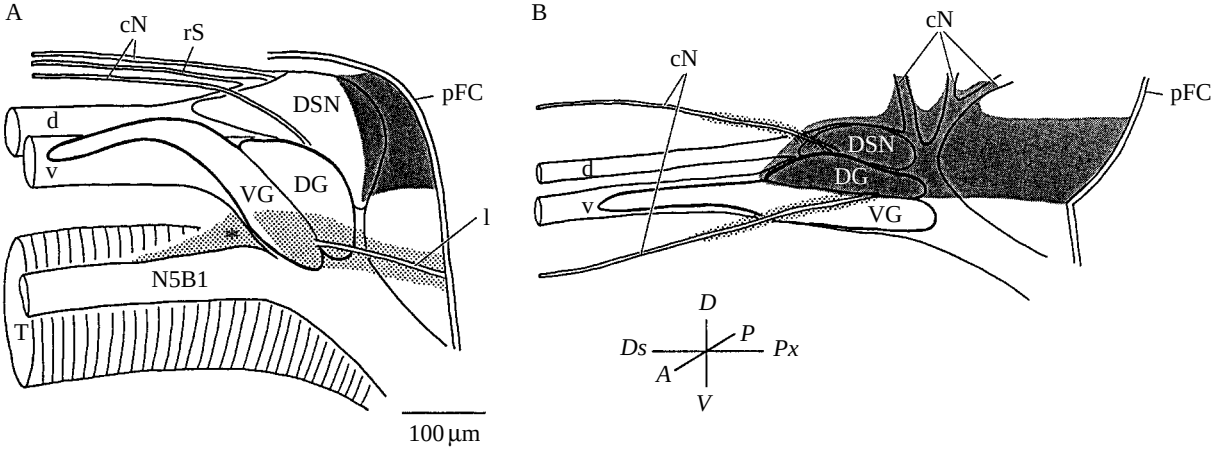

Fig. 5. Semi-schematic drawings (anterior view) of the connective tissues binding the scoloparia with surrounding structures. (A) Prothoracic FCO; (B) metathoracic FCO. VG, ventral group; DG, dorsal group; DSN, dorsal scoloparium neurones. Shaded regions show the connective tissues (dark shade, stiff connection; light shade, elastic connections). An asterisk indicates the connective tissue supporting the fork of the FCO nerve and N5B1. l, ligament; pFC, proximal femoral cuticle; T, large longitudinal trachea; d, dorsal ligament; v, ventral ligament; cN, cuticular nerve; rS, receptor strand of the strand receptor. For other abbreviations, see Fig. 1.

were observed to connect scoloparia with the cuticle. In one region, connective tissue surrounding the ligament (lightly shaded area without asterisk) loosely connected the anterior surface of the ventral scoloparium with the anterior proximal cuticle wall; in another region, connective tissue (darkly shaded area) firmly connected both the dorsal group (not shown) and the dorsal scoloparium with the proximal cuticle.

The connective tissues in the metathoracic FCO are shown in Fig. 5B. The connection pattern was somewhat different from that seen in the prothoracic FCO. The ventral group was free from the surrounding structures, and the dorsal group and the dorsal scoloparium were firmly connected by the common connective tissue (darkly shaded area) not only to the proximal cuticle but also to the anterior hypodermis (not shown) of the femur.

Displacement of FCO neurones by a change in F-T joint angle

The positional change of the retrogradely stained neurones was measured while the tibia was held at different angles. This experiment does not give a direct estimate of the specific physiological properties but provides information on the relative elasticity of the connective tissues embedding the three neural groups as examined by Nowel *et al.* (1995). Fig. 6A shows the results for the prothoracic FCO neurones. At 160 ° (full joint extension), somata were positioned at 0 µm by definition. Neurones in the ventral group were gradually displaced distally more than those in other groups when the F-T joint was flexed. Within the ventral group, the distal smaller neurones (Fig. 6A, a) were displaced by 100±9 µm (N=6) at 0 °, while proximal larger neurones (Fig. 6A, c) were displaced by approximately 23±18 µm (N=6). Intermediate neurones (Fig. 6A, b) showed an intermediate amount of movement (56±22 µm, N=6). This is partly because the ventral group has no strong connection with the proximal cuticle (see Fig. 5) and because the distally located neurones were embedded in the soft connective tissue. In contrast, neurones in the dorsal group (Fig. 6A, d), and those in the dorsal scoloparium (Fig. 6A, e) located at nearly the same level as the proximal larger neurones (Fig. 6A, c), were displaced much less: 5±10 µm (N=6) for group d and 5±2 µm (N=6) for group e. This is probably because both the dorsal group and the dorsal scoloparium have a strong connection with the proximal cuticle (see Fig. 5). A similar tendency was observed in the metathoracic FCO (Fig. 6B). However, here the displacement in proximal neurones of the ventral group (Fig. 6B, c) was greater than in the prothoracic FCO. This is partly because of the difference in the curvature of the neural chain (Fig. 6A,B, insets): neurones in the metathoracic FCO are pulled distally during tibial flexion, while those in the prothoracic FCO are pulled distally and dorsally.

In summary, neurones in the ventral group are markedly displaced by pulling the apodeme, the degree of displacement depending upon their location within the neural chain, while neurones in the dorsal group and the dorsal scoloparium are similar to each other in their mechanical properties.

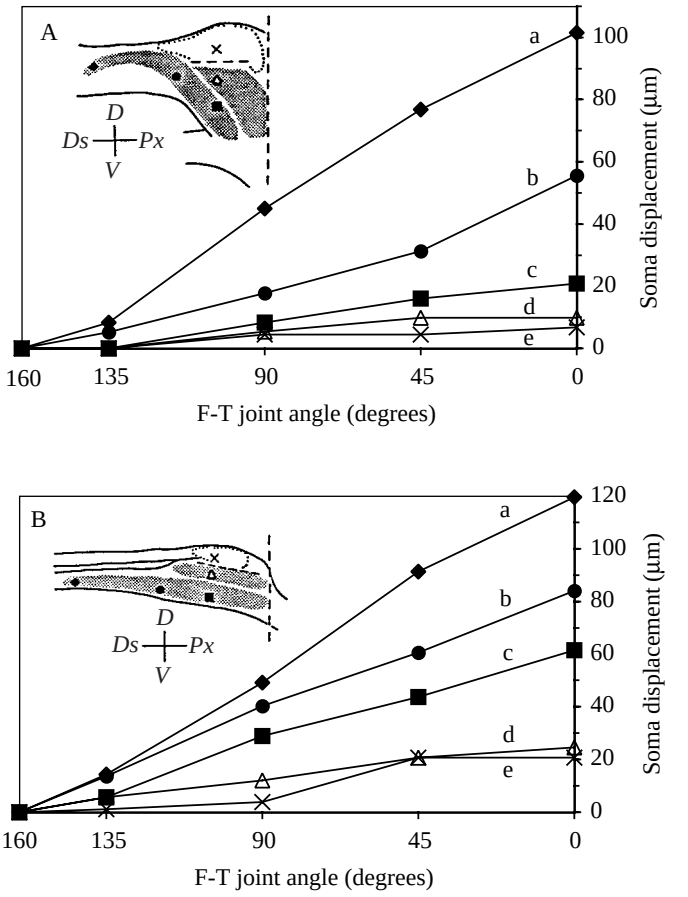

Fig. 6. Displacement of neurones at different F-T joint angles. The degree of movement of three neural groups is shown for (A) the prothoracic femoral chordotonal organ (FCO) and (B) the metathoracic FCO. Measurements were made successively at different F-T joint angles: 0 °, 45 °, 90 °, 135 ° and 160 °. Symbols on the insets shows the locations of the neurones sampled. Six neurones were examined at each site in six preparations. Shaded areas show the ventral and dorsal groups in the ventral scoloparium and the dotted outline indicates the dorsal scoloparium. In A, ◆ is one of the ventral group neurones located approximately 380 µm from the reference line (see vertical dashed line in the inset) at 160 °, ● is one of the ventral group neurones located approximately 225 µm from the reference line, ■ is a ventral group neurone located approximately 110 µm from the reference line at 160 °, △ is one of the dorsal group neurones located at the same level as ■ and × is one of the dorsal scoloparium neurones located at the same level as ■. In B, ◆ is one of the ventral group neurones located approximately 400 µm from the reference line at 160 °, ● is one of the ventral group neurones located approximately 235 µm from the reference line, ■ is one of the ventral group neurones located approximately 140 µm from the reference line, △ is one of the distally located neurones in the dorsal group located at nearly the same level as ■ and × is one of the dorsal scoloparium neurones located at the same level as ■.

Central projection of FCO neural groups

All the sensory neurones of the three neural groups projected to the ipsilateral hemiganglion in the thorax through N5. Their projection patterns and projection areas were essentially

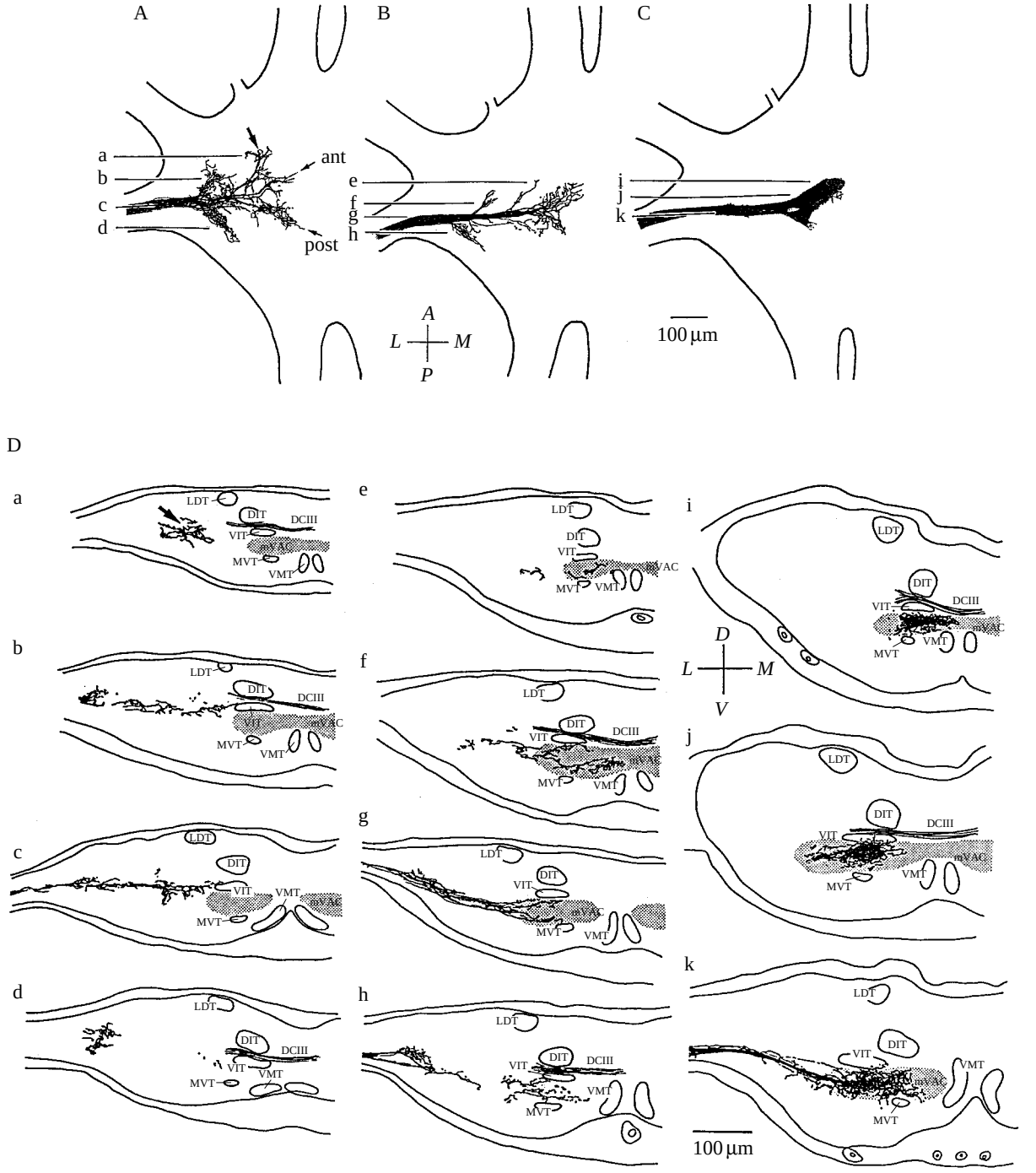

Fig. 7. *Camera lucida* drawings of central projections of the three neural groups. (A) Ventral group. ant, anterior medial bundle; post, posterior medial bundle. The arrow indicates the Y-shaped branches. (B) Dorsal group. (C) Dorsal scoloparium neurones. The ganglion is viewed from the ventral surface. a–k show the sectioning levels corresponding to those in D. (D) Transverse sections (18 µm) of the three ganglia (A–C). Shaded area shows the ventral association centre (VAC). DIT, dorsal intermediate tract; DCIII, dorsal commissure III; LDT, lateral dorsal tract; MVT, median ventral tract; mVAC, medioventral association centre; VIT, ventral intermediate tract; VMT, ventral median tract.

similar in the prothoracic (Fig. 7) and metathoracic (Fig. 8) FCOs. The axons of hair sensilla on the cuticle innervated by the cuticular nerve sometimes intermingled with those of the FCO neurones. However, they were easily discriminated because the former terminated in the ventralmost ventral association centre (vVAC), which was completely separated from the FCO projection fields.

After entering the thoracic ganglion, the axons of the ventral group in the ventral scoloparium (Figs 7A, 8A) gave off abundant branches in the dorso-lateral region (Figs 7Dd, 8Dd),

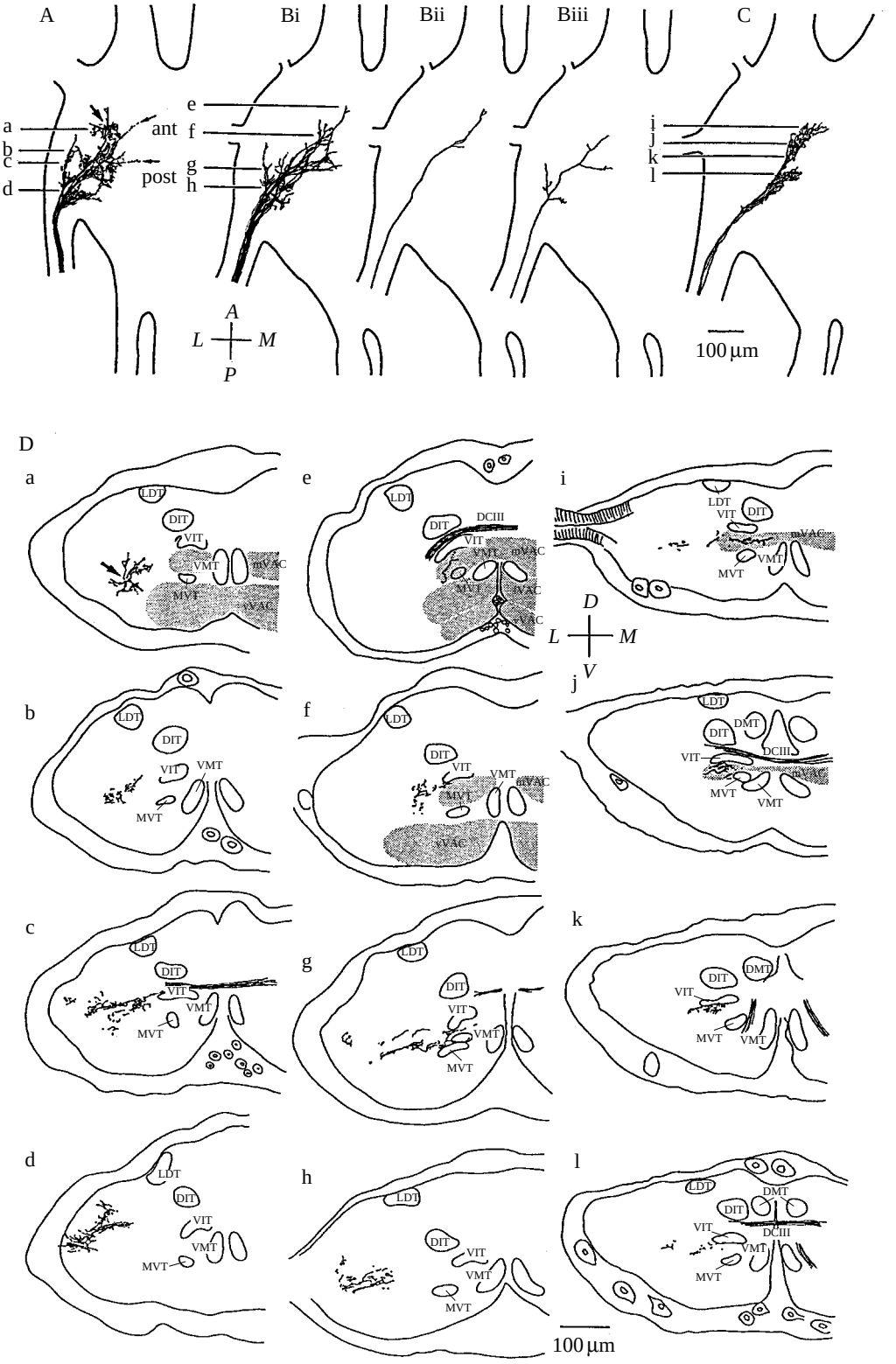

Fig. 8. *Camera lucida* drawings of central projections of the three neural groups. (A) Ventral group. ant, anterior medial bundle; post, posterior medial bundle. The arrow indicates the Y-shaped branches. (Bi) Dorsal group; (Bi,Bii) branching patterns of the two single axons. (C) Dorsal scoloparium neurones. The ganglion is viewed from the ventral surface. a–l show the sectioning levels corresponding to those in D. (D) Transverse sections (18 μm) of the three ganglia A, Bi and C. The shaded area shows the ventral association centre (VAC). DMT, dorsal median tract; lVAC, lateral VAC; vVAC, ventral VAC. For other abbreviations, see Fig. 7.

which corresponds to the lateral association centre (LAC) in the locust (Pflüger *et al.* 1988). The main neurites gave off two further bundles, termed the anterior (ant) and posterior (post) medial bundles in the locust (Matheson, 1992). The anterior medial bundle gave off well-developed Y-shaped branches (arrows in Figs 7A,Da, 8A,Da). These two bundles terminated in the regions between the ventral intermediate tract (VIT) and the dorsal intermediate tract (DIT) (Figs 7Db,c, 8Da,c). No terminals were observed in the medioventral association centre (mVAC).

For the dorsal group (Figs 7B, 8Bi), the axons gave off sparse dorso-lateral branches (Figs 7Dh, 8Dh). The main neurites projected further medially to the mVAC in which terminal arborizations were observed mainly in the marginal region of the mVAC (Figs 7De–g, 8De,f). On the basis of single axon tracing, at least two types of projection could be observed in the metathoracic FCO. The first type (N=11) ran slightly more ventrally with no larger branches (Fig. 8Bii). The second type (N=9) had a few dorso-lateral branches and medial bifurcations (Fig. 8Biii).

In contrast, the axon terminals of the dorsal scoloparium neurones (Figs 7C, 8C) formed a crescent-shaped region near the midline exclusively in the mVAC (Figs 7Di–k, 8Di,j). They occasionally crossed the midline by approximately 20 μm. In the metathoracic FCO, the terminals were also observed in the region ventral to the VIT (Fig. 8Dk,l).

Discussion

In the cricket, the pro- and metathoracic FCOs consist of two partly fused scoloparia, the ventral scoloparium and the dorsal scoloparium in the proximal femur. The arrangement of neuronal somata in the scoloparium, the joint-flexion-dependent positional changes of the somata and the central projections of the neurones were similar in the pro- and metathoracic FCOs.

Comparison of the FCO structure between species

It has been reported that partly fused scoloparia similar to those in *Gryllus bimaculatus* are present in another species of cricket, *Acheta domesticus* (Nowel *et al.* 1995), in a tettigoniid (Theophilidis, 1986), in the weta (Matheson and Field, 1990) and in the stick insect (Füller and Ernst, 1973). In the locust, however, two scoloparia in the pro- and mesothoracic FCOs are completely separated into the distal scoloparium and proximal scoloparium and are innervated by different nerve bundles (Burns, 1974; Field and Pflüger, 1989).

In the present study, we have shown that the ventral scoloparium of the cricket *Gryllus bimaculatus* consisted of larger neurones projecting broadly to the lateral regions in the thoracic ganglion. Some terminal arborizations were observed in a restricted region of the medioventral association centre (mVAC), which is known to be the auditory neuropile. In contrast, the dorsal scoloparium consisted of smaller neurones projecting exclusively to the mVAC. In parallel with this anatomical design in the cricket, the locust distal scoloparium consists of larger neurones which project broadly to the lateral regions, while the proximal scoloparium consists of smaller neurones projecting exclusively to the mVAC. We therefore conclude that the ventral scoloparium in the cricket may be homologous with the distal scoloparium in the locust, while the dorsal scoloparium in the cricket is homologous with the proximal scoloparium in the locust.

Dorsal scoloparium neurones

The cricket dorsal scoloparium consists of small neurones clustering in close proximity to the femoral wall. These neurones were hardly displaced when the F-T joint was flexed. The axons project exclusively to the mVAC, forming a crescent shape. In the locust, neurones in the proximal scoloparium (corresponding to the dorsal scoloparium in the cricket) are known to respond strongly to apodeme vibration with a maximum sensitivity of 300 Hz (Field and Pflüger, 1989). In the crickets *Gryllus campestris* and *Gryllus bimaculatus* (Eibl and Huber, 1979) and the grasshopper *Decticus verrucivorus* (Kalmring *et al.* 1978), the auditory organ (tympanal organ) neurones have been shown to project their axons to the ventral region of the mVAC. These facts suggest the possibility that the dorsal scoloparium neurones might be concerned with vibration reception.

Unlike the locust, the dorsal scoloparium (corresponding to the proximal scoloparium in the locust pro- and mesothoracic FCOs) is also found in the cricket metathoracic FCO, which contains far fewer neurones than the prothoracic FCO. It is possible that the dorsal scoloparium has evolved in the metathoracic legs as a result of their specialization for jumping. However, this speculation can only be substantiated by extensive studies using many different species.

It has been reported that, in *Acheta domesticus*, dorsal scoloparium neurones (15–25 μm) in the metathoracic FCO are larger in soma diameter than ventral scoloparium neurones (13–16 μm) (Nowel *et al.* 1995). This is not in agreement with our present finding that dorsal scoloparium neurones (9.0±1.1 μm) are smaller than ventral scoloparium neurones (ventral group, 10.2±4.3 μm; dorsal group, 12.1±2.1 μm). Since both dorsal group and dorsal scoloparium neurones are situated very close to one another and are covered by common connective tissue (see Fig. 5B), and because Nowel *et al.* (1995) did not inspect the dendritic intrusion into the ligaments, they may have included the dorsal group neurones of the ventral scoloparium among the dorsal scoloparium neurones and thus arrived at a different result. However, the possibility of a species difference cannot be excluded.

Ventral scoloparium neurones

Ventral group

The ventral group consisted of several chain-like clusters of neurones arranged sequentially from large to smaller, distally situated neurones. Neurones in this group were easily displaced during F-T joint flexion, with the distal somata being displaced more than the proximal somata. Furthermore, their dendrites are likely to stretch when their somata are displaced distally. These properties probably represent a mechanical basis for a range fractionation function derived from the structure of the ligaments: attachment cells connecting the cuticular core with the distally located neurones develop higher tension than those associated with the more proximally located ones since the coiled cuticular core in the ventral ligament begins to unfold from the distal end during flexion (Nowel *et al.* 1995). Such a differential tensioning system has parallels with the ventral

ligament in the locust metathoracic FCO (Field, 1991; Shelton *et al.* 1992). In the locust, directionally sensitive neurones in the distal scoloparium respond to slow and large displacement of the apodeme, and mediate the resistance reflex in the femoral muscles (Field and Pflüger, 1989). A similar observation was made for the ventral scoloparium neurones of the stick insect (Büschges, 1994).

Examination of the central projection of the neurones showed that the ventral group axons gave off branches abundantly in the lateral regions of the ganglion, including the lateral association centre where medially oriented axons (anterior and posterior medial bundles) extended towards the region between the dorsal intermediate tract and the ventral intermediate tract. The lateral association centre has been recognized as a reflex centre in the metathoracic ganglion because many motoneurones and spiking interneurones, which respond to mechanical stimulation of the FCO, have their branches within it (Burrows, 1988; Burrows *et al.* 1988). In the locust, a similar projection was reported for the distal scoloparium neurones of the mesothoracic FCO (Field and Pflüger, 1989) and metathoracic FCO (Pflüger *et al.* 1988; Matheson, 1992). These results suggest the possibility that neurones of the ventral group of the ventral scoloparium might be associated with the resistance reflex.

Dorsal group

This group, whose somata were relatively regular in size and concentrated in the dorso-proximal region of the ventral scoloparium, is newly described in this study. These neurones showed little displacement in response to F-T joint movement. These characteristics suggest that they resemble dorsal scoloparium neurones rather than the ventral group of ventral scoloparium neurones.

The central projection of the dorsal group neurones showed an intermediate pattern between those of the ventral group neurones and the dorsal scoloparium neurones. That is, their axons terminated in both the dorso-lateral regions and the peripheral region inside the medioventral association centre in the thoracic ganglion. This arborization pattern seems to be close to that of some sensory neurones in the cricket subgenual organ, which responds to substratum vibration (Eibl and Huber, 1979; Esch *et al.* 1980). In the locust, neurones in some thoracic chordotonal organs (anterior chordotonal organ and myochordotonal organ) are known to have axonal branches at least in the ventral margin of the medioventral association centre (Bräunig *et al.* 1981; Pflüger *et al.* 1988). However, the distal scoloparium (corresponding to the ventral scoloparium in the cricket) neurones in the locust have no axonal branches in the medioventral association centre (Field and Pflüger, 1989; Matheson, 1992). Some single axons of the dorsal group in the cricket had no large branches (see Fig. 8Bii), thus resembling projections of some neurones in the dorso-proximal region of the metathoracic FCO in the locust (Matheson, 1992) that responded to acceleration and strongly to leg position (Matheson, 1990). From these observations, the dorsal group in the cricket may contain neurones of intermediate sensitivity (e.g. to low-frequency vibration) between fast, small apodeme movement reception (of high-frequency vibration) in the dorsal scoloparium and slow, large apodeme movement reception in the ventral group of the ventral scoloparium.

Taken together, the above points suggest functional differentiation of the FCO to an even greater extent than previously thought. Our present findings provide the basis for future neurophysiological studies of the cricket FCO.

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