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Parallel Somatotopic Maps of Gustatory and Mechanosensory Neurons in the Central Nervous System of an Insect

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ABSTRACT

Relatively little is still known about the sense of taste, or contact chemoreception, compared with other sensory modalities, despite its importance to many aspects of animal behaviour. The central projections of the sensory neurons from bimodal contact chemoreceptors (basiconic sensilla) were compared with those from mechanosensory tactile hairs located on similar regions of the middle leg of the locust. Basiconic sensilla are multiply innervated, containing one mechanosensory and several chemosensory neurons, whereas tactile hairs are innervated by a single mechanosensory neuron. We show that the sensory neurons from tactile hairs form a complete 3-dimensional somatotopic map in the mesothoracic ganglion. Sensory neurons from hairs located on the coxa projected to a region near the midline of the ganglion with neurons from hairs located on progressively more distal parts of the leg arborizing in successively more lateral regions of neuropil. All the neurons from basiconic sensilla, both mechanosensory and chemosensory, also projected in a similar, strictly somatotopic, manner, and the arbors from these neurons overlapped considerably with those from tactile hairs on equivalent parts of the leg to form a continuous region. Thus, the position of a receptor on the leg is preserved in the central nervous system not only for the mechanosensory neurons from both tactile hairs and basiconic sensilla but also for chemosensory neurons. We could observe no anatomical features or small differences in projection region between sensory neurons from individual basiconic sensilla consistent with differences in modality. J. Comp. Neurol. 425:82–96, 2000.

Indexing terms: chemoreception; gustation; mechanoreception; sensory neuron; locust

A characteristic feature of the nervous systems of many animals is the maps formed by the consistent and orderly projections of sensory neurons. These maps may represent the location of sensory receptors on or in the body, and/or coding properties of the sensory neurons. For example, the central projections of mechanosensory neurons innervating hairs on the limbs of invertebrates (Zill et al., 1980; Murphey, 1981; Pflüger et al., 1981; Johnson and Murphey, 1985; Levine et al., 1985; Kent and Levine, 1988; Peterson and Weeks, 1988; Newland, 1991) and vertebrates (Brown et al., 1977, 1980) form somatotopic maps in which the spatial location of the receptor on the limb is preserved. The central projections of sensory neurons from the eyes are arranged retinotopically (Strausfeld, 1976), whereas in the auditory system the sensory neurons form a tonotopic map (Oldfield, 1982; Römer, 1983; Römer et al., 1988). In the olfactory systems of both invertebrates and vertebrates there is an odotopic mapping of sensory neurons into compartments within the primary olfactory neuropils of the brain (Hildebrand and Shepherd, 1997).

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In contrast to these sensory systems, comparatively little is known about the gustatory chemosensory system, in terms of either the organisation of the chemosensory afferents or the coding and processing of their signals by central neurons. The principal contact-chemosensory organs of orthopteroid insects are bimodal receptors that contain 1 mechanosensory neuron and up to 12 chemosensory neurons (Blaney and Chapman, 1969; Kendall, 1970; Klein, 1981), depending on the species and location on the body. These sense organs, called basiconic sensilla in locusts, are distributed over the mouthparts, body, and limbs (Chapman, 1982) and play a vital role in food acceptance and rejection, avoidance behaviour, and the selection of mates and appropriate egg laying sites (Ma and Schoonhaven, 1973; Dethier, 1976; White and Chapman, 1990; Städler et al., 1995; Newland, 1998). Although there have been many studies on the sensory physiology of these contact chemoreceptors, we still know little about how information from sampled chemicals is integrated in the nervous system, and even the precise organisation of chemosensory neurons in the central nervous system is unclear. Murphey et al. (1989a) suggested that there is a modality-specific segregation of presumed chemosensory and mechanosensory afferents within the central nervous system in much the same way as there is a segregation of exteroceptive and proprioceptive sensory neurons (Burrows, 1987; Pflüger et al., 1988). This was hypothesized by comparing the sensory projections of bimodal sensilla with those from tactile hairs. Edgecomb and Murdock (1992) made a similar claim on the basis of a spatial segregation of large- and small-diameter axons, for neurons from bimodal taste receptors on the labellum of *Phormia*. This contrasted with an earlier study that found no anatomical segregation on the basis of modality in the same species (Yetman and Pollack, 1986).

It has been known for some time that spiking local interneurons receive convergent monosynaptic exteroceptive inputs both from the purely mechanosensory tactile hair afferents and from the mechanosensory afferents innervating basiconic sensilla (Newland and Burrows, 1994). As the sensory neurons from tactile hairs form a somatotopic map (Newland, 1991) and the input branches of spiking local interneurons are arranged along this map according to their particular receptive fields (Burrows and Newland, 1993), it is possible that at least the mechanosensitive afferents from basiconic sensilla could follow a similar somatotopic organisation. It is now also known that these same spiking local interneurons also receive convergent inputs from at least one of the chemosensory neurons innervating basiconic sensilla (Newland, 1998, 2000). This further implies that some chemosensory neurons may also project to the same or adjacent regions in the ventral neuropil as exteroceptive sensory neurons. The aim of the present study was to determine whether this emerging physiological convergence has any anatomical correlate by explicitly examining the organisation of the sensory neurons from both tactile hairs and basiconic sensilla over the surface of the middle leg.

MATERIALS AND METHODS

Experiments were performed on adult male and female desert locusts, *Schistocerca gregaria* (Forskål), taken 24–72 hours after their final moult from crowded colonies at the Universities of Southampton and Cambridge. Animals were restrained either dorsal or ventral side uppermost with modelling clay in 14-cm-diameter plastic dishes.

Cobalt staining

The method used to stain the tactile hair afferents of locusts has been described in detail (Newland, 1991). Briefly, retrograde stains of the sensory neurons innervating tactile hairs on the middle leg were made by surrounding an individual hair with petroleum jelly to form a small well. A 6% solution of cobalt hexamine was placed into the well and the hair cut to half its length, under the surface of the dye, before the well was sealed with more petroleum jelly. Locusts were then incubated for 5–10 days at 4°C to allow the cobalt to diffuse throughout the neuron. Subsequently, animals were dissected from the ventral surface, the mesothoracic ganglia removed, and the cobalt was precipitated as the sulphide (Pitman et al., 1972). Ganglia were then fixed in 5% formaldehyde and intensified with silver (Bacon and Altman, 1977).

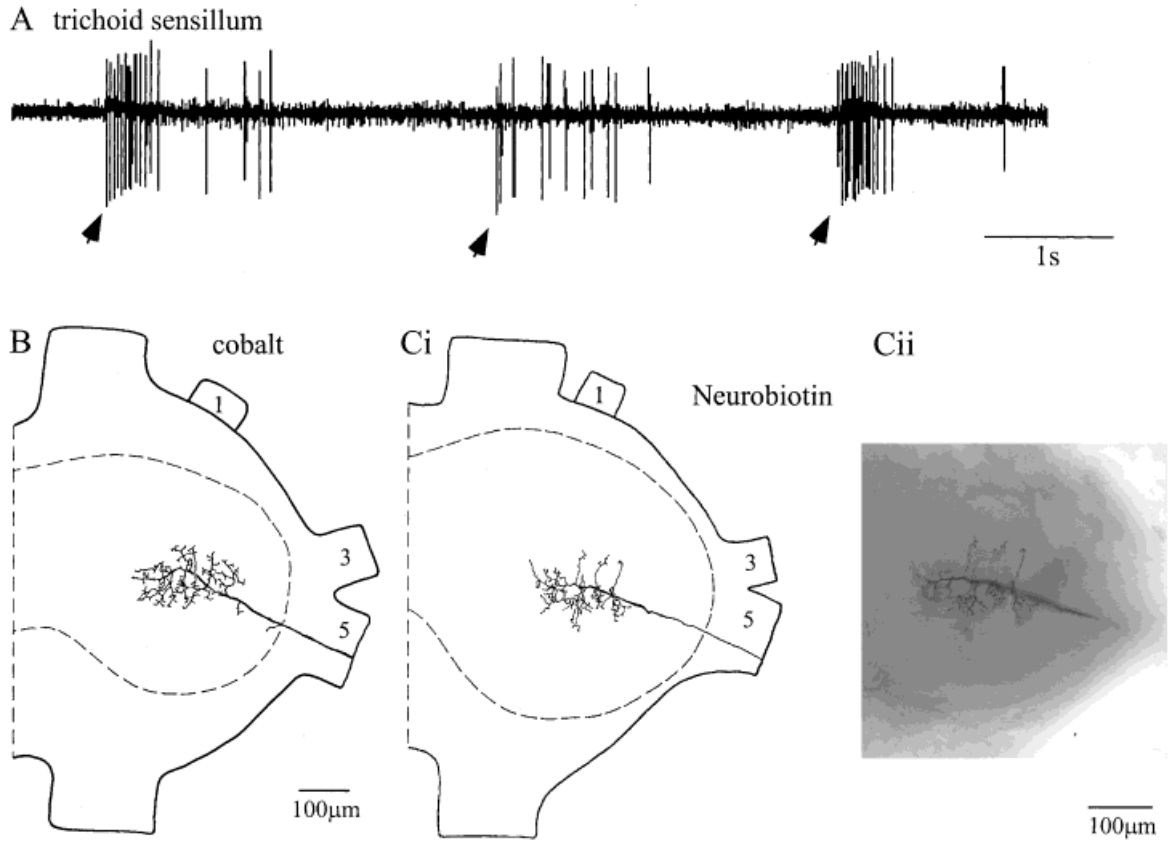
Neurobiotin staining

Staining the sensory neurons from basiconic sensilla with cobalt proved to be difficult (Burrows and Newland, 1994), probably because of the small diameter of their axons. We therefore used neurobiotin to stain these sensilla. Individual basiconic sensilla were cut with a razor blade at the level of the cuticle and surrounded by a well of petroleum jelly. Neurobiotin (3%) in deionized water (Vector, Burlingame, CA) (Kita and Armstrong, 1991) was placed into the wells, which were then sealed and the preparations incubated as described above.

After incubation and dissection, mesothoracic ganglia were fixed in 10% formalin for 20 minutes, dehydrated in an ascending alcohol series, and washed in xylene for 30 minutes. They were then rehydrated and rinsed twice in phosphate-buffered saline (PBS; pH 7.2, 10 minutes each). Next, the ganglia were transferred to a solution of 1 mg collagenase (type IV; Sigma-Aldrich, Poole, UK) and 1 mg hyaluronidase (Sigma, type II) in 1ml PBS, and incubated at 37°C for 1 hour, and subsequently washed for 15-minute periods twice in PBS and three times in PBS containing 0.5% Triton X-100 (Sigma-Aldrich). They were then transferred to a Vectastain (Vector) ABC reagent [1 drop reagent A (Avidin DH) and 1 drop reagent B (biotinylated horseradish peroxidase in 5 ml PBS)] for incubation overnight at room temperature.

Following incubation the ganglia were washed in three changes of PBS containing 0.5% Triton X-100 and once in PBS (15 minutes each) before being transferred to 1 ml PBS containing 0.3 mg diaminobenzedrine hydrochloride (DAB; Sigma). Four microliters of 30% hydrogen peroxide was added to the solution, and the reaction was observed until the ganglia turned a deep brown colour, at which point the reaction was terminated by transferring the ganglia through two changes of PBS (5 minutes). The preparations were then dehydrated in an ascending alcohol series and cleared in methyl salicylate for wholemount viewing.

Cobalt- and neurobiotin-stained afferents were drawn with the aid of drawing tubes fixed to either a Zeiss Axiophot or a Nikon E-400 compound microscope from both wholemounts, and approximately 500 μm-thick transverse sections were cut with a razor blade. The nomenclature used to describe the various regions of the neuropil of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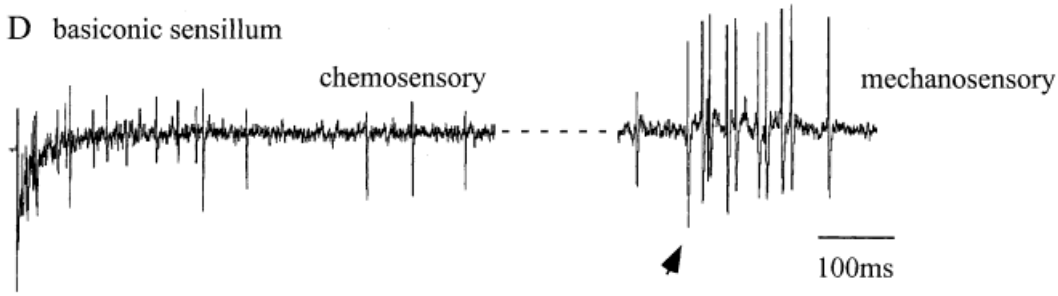
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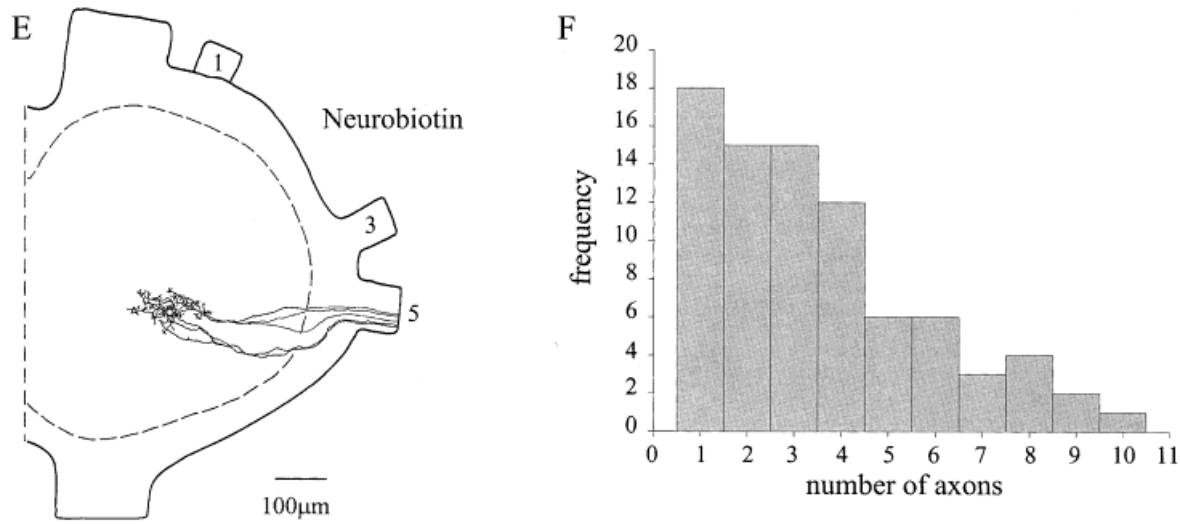
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0 2 4 6 8 10 12 14 16 18 20

Figure 1

the mesothoracic ganglia is based on Tyrer and Gregory (1982) and Pflüger et al. (1988). Data are based on 57 successful cobalt stains of tactile hair afferents from 246 locusts and 83 successful neurobiotin stains of afferents from individual basiconic sensilla from 210 locusts.

Electrophysiology

The tip-recording technique (Hodgson et al., 1955) was used to record from the sensory neurons innervating both the tactile hairs (trichoid sensilla) and contact chemoreceptors (basiconic sensilla). Before recording, tactile hairs were cut to approximately half their length, but basiconic sensilla were left intact. Blunt glass recording microelectrodes containing 250 mM sodium chloride were then placed directly over the tips of the sensilla. The salt solution in the electrodes evoked spikes in some of the chemosensitive neurons, and movements of the electrode, which deflected the shafts of the sensilla, induced spikes in mechanosensory neurons (Newland and Burrows, 1994). The same electrode was therefore used to evoke and record simultaneously the spikes of both the mechano- and chemosensory afferents.

RESULTS

Innervation of tactile hairs and basiconic sensilla

Tactile hairs on the mesothoracic leg are each innervated by a single mechanosensory neuron, as indicated by the presence of spikes with a single amplitude only in tip recordings from these sensilla following deflection of the hair-shaft (Fig. 1A). Similarly, only single sensory neurons were stained in the mesothoracic ganglion in backfills from tactile hairs using either cobalt hexamine (Fig. 1B) or neurobiotin (Fig. 1Ci, ii). Conversely, spikes with several distinct amplitudes were elicited by placing electrodes containing 250mM sodium chloride over basiconic sensilla (Fig. 1D), indicating that more than one chemosensitive neuron was activated by the salt solution. In the example shown, the spike frequency of the chemosensory neurons decreased rapidly with time. Deflecting the shaft of the basiconic sensillum 3 seconds later evoked a further burst of larger amplitude action potentials from a mechanosensitive neuron (Fig 1D). Over 78% (n = 83) of successful backfills from individual basiconic sensilla had two or more axons entering the ganglion (Fig. 1E,F). Each basiconic sensillum on the leg of a locust is thought to be innervated by one mechanosensory neuron and three to four chemosensory neurons, as assessed by physiological and anatomical methods (Chapman, 1982; White and Chapman, 1990). The number of axons stained in the central nervous system revealed by backfills from single basiconic sensilla on the leg was variable (Fig. 1F). This is not likely to be a true reflection of the number of sensory neurons within a basiconic sensillum or of variability between them but is probably indicative of the capriciousness of the staining method. It is clear from the results, however, that all tactile hairs were singly innervated and unimodal, but that basiconic sensilla were multiply innervated and bimodal.

Projections of sensory neurons innervating tactile hairs

Backfills were performed on selected tactile hairs located on the dorsal surface of each of the leg segments; coxa (n = 5), trochanter (n = 3), femur (n = 21), tibia (n = 11), and tarsus (n = 9) and from the lateral thorax (epimeron, n = 8) immediately dorsal to the coxa, to determine the organisation of afferents from tactile hairs along the entire proximo-distal axis of the leg. Figure 2 illustrates representative projection patterns of sensory neurons from each of these locations. The total area of arborization occupied by sensory afferents from over the entire leg occupies a region of ventral neuropil extending from near the midline to the lateral edge of the ganglion (Fig. 2A–F, stippled regions). This region lies across the approximate middle of the anterior-posterior axis of the ganglion at its most medial and slopes gradually posteriorly toward the lateral edge of the ganglion (Figs. 2A–F, 5). The arborizations of neurons from sensilla located on any particular part of the leg occupy only a small part of this region. Sensory neurons from sensilla located on the epimeron (Fig. 2A) and proximal leg (Fig. 2B,C) occupy the more medial neuropil, and the arborizations of neurons from sensilla on other parts of the leg (Fig. 2D–F) occur progressively more laterally as the locations of the sensilla move distally along the leg.

Thus, although there is some overlap between the arborizations of sensory neurons from adjacent parts of the leg, there is a systematic displacement of the arborizations laterally in the ganglion from sensilla located on successively more distal parts of the leg (Fig. 2G). Axons from neurons innervating hairs on the thorax enter the ganglion through nerve 4 (Fig. 2A) and those innervating hairs on the coxa through nerve 3 (Fig. 2B) whereas those innervating the other regions of the leg all enter through nerve 5 (Fig. 2C–F). The paths of the axons through the ganglion to the arborization region were quite variable, much more so than the final destination. In the examples shown, the axon from the femoral hair (Fig. 2D) runs anterior of nerve 5 across the ganglion before curving round to a more posterior arborization, whereas that from the tibial hair (Fig. 2E) runs around the posterior edge of the neuropil before curving forward and arborizing, but examples exist of axons taking the opposite routes to those illustrated.

Fig. 1. Innervation of trichoid (tactile hairs) and basiconic (contact chemoreceptors) sensilla. **A:** Deflecting the cut shaft of a trichoid sensillum (arrows) evokes bursts of action potentials of a single amplitude, indicating that the sensillum is innervated by a single mechanosensory neuron. Backfilling cut tactile hairs on the dorsal tarsus of the mesothoracic leg with either cobalt (**B**) or neurobiotin (**Ci**; photograph of the same ganglion, **Cii**) results in the staining of a single sensory neuron in the mesothoracic ganglion. In this and subsequent drawings of the mesothoracic ganglion, anterior is to the top, the outline of the neuropil area is indicated with a dashed line, and the ganglionic midline is indicated by a vertical dashed line. Only the right halves of the ganglion are shown. **D:** Placing an electrode filled with 250 mM sodium chloride over a basiconic sensillum on the dorsal tibia of the mesothoracic leg evokes a burst of action potentials of different amplitudes. Subsequent displacement of the shaft of the sensillum (arrow, right side) evokes a burst of action potentials of a much larger amplitude from a single mechanosensory neuron. **E:** Backfilling a basiconic sensillum on the dorsal surface of the tarsus of the mesothoracic leg results in the staining of five sensory neurons that project to a ventral region of neuropil. **F:** The number of centrally stained axons resulting from backfilling a single basiconic sensillum was variable (n = 82).

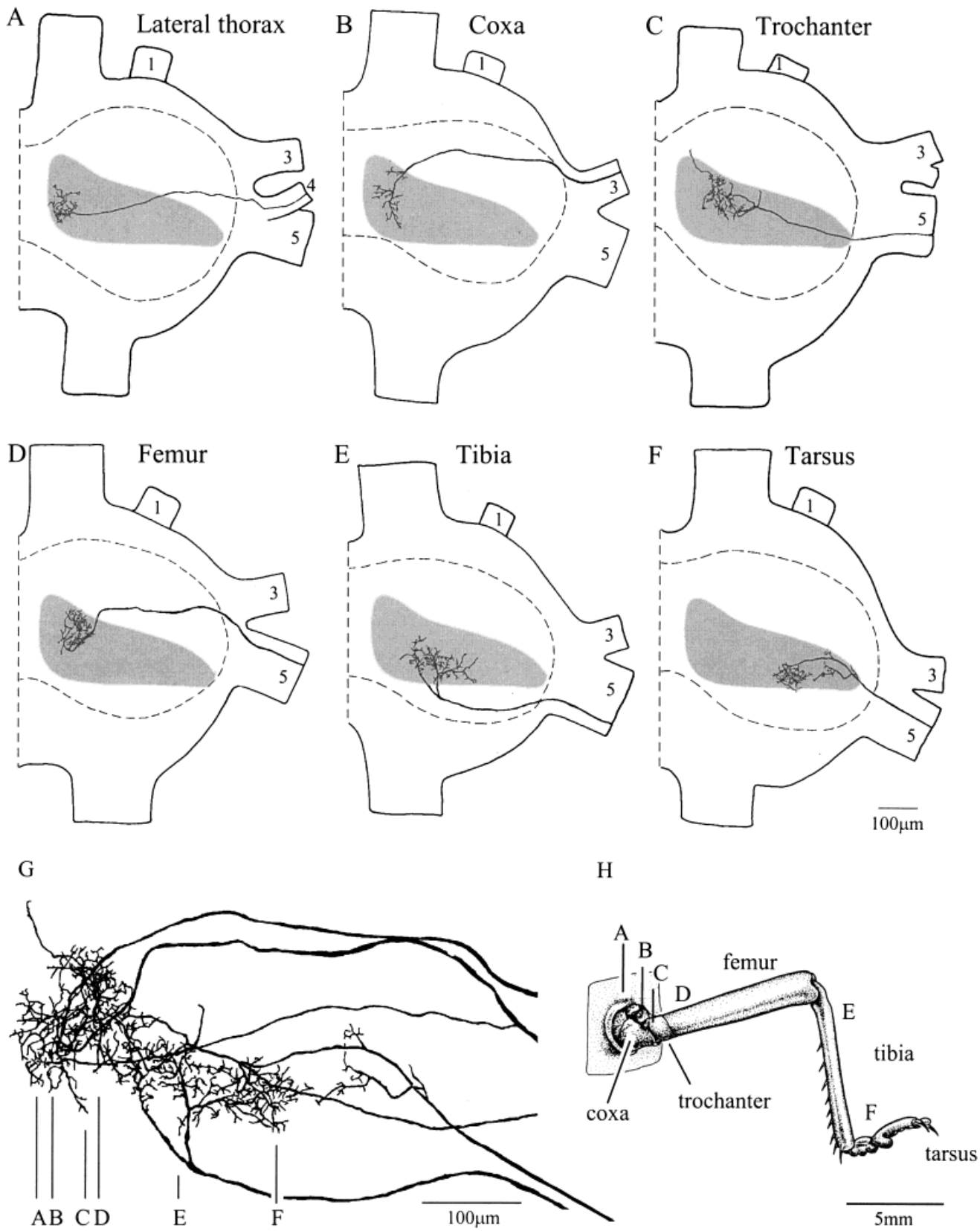

Fig. 2. Central projection of sensory neurons from tactile hairs along the proximo-distal leg axis. **A:** A single sensory neuron from a hair on the thorax projects to a central region of ventral neuropil. **B:** A sensory neuron from a hair on the dorsal coxa overlaps with that from the thorax, although some of its branches are more lateral. **C:** A sensory neuron from a hair on the dorsal trochanter overlaps with the coxal hair projections but is again generally more lateral. The central projections of sensory neurons from hairs on the femur (**D**), tibia (**E**), and tarsus (**F**) each in turn project more laterally, so that those from a tarsal hair are closest to the lateral edge of neuropil. **G:** Superimposing the sensory neurons from the different positions on the leg (**H**) shows that there is a systematic change in projection position based on the spatial location of the hair on the leg such that proximally located hairs send their sensory projections most medially in the ganglion whereas distally located hairs have their projections located most laterally in the ganglion. The stippled area in the ganglion corresponds to the total area occupied by the mechanosensory neurons from the tactile hairs.

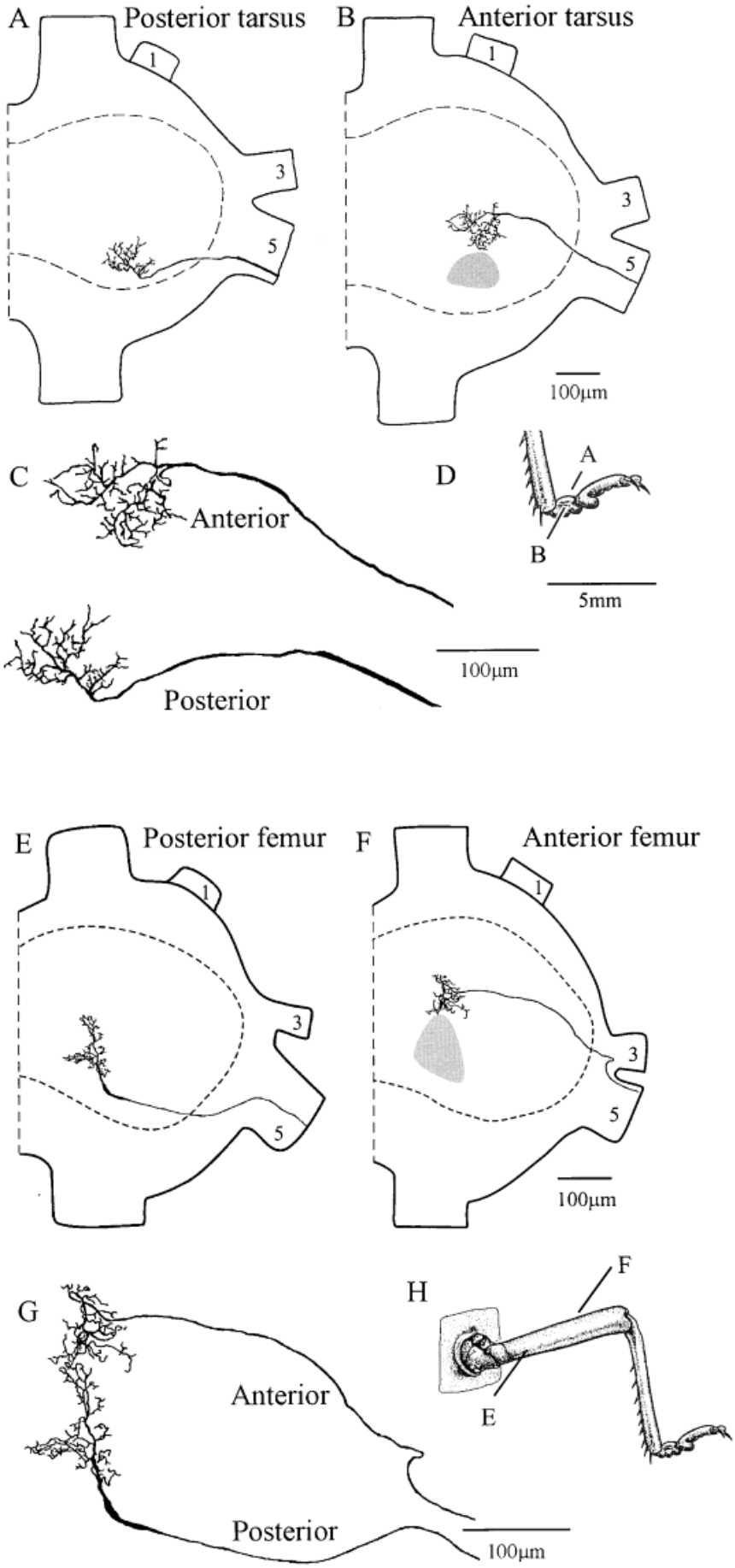

Figure 3 (Overleaf)

Mücke and Lakes-Harlan (1995) could find no systematic differences between sensory projections from hairs on the anterior and posterior surfaces of the middle leg of the locust, in contrast to the hind leg (Newland, 1991). To test this, we selected hairs on the anterior and posterior surfaces of the leg and stained these with cobalt hexamine (n = 16) and examples from two locations along the leg are shown in Figure 3. The stained axon from a tactile hair on the posterior tarsus entered the ganglion in the posterior part of nerve 5 and then travelled along the posterior edge of the neuropil before turning inward, projecting to an area of neuropil lateral to the connectives (Fig. 3A). The stained axon from an anterior tarsal hair projected more anteriorly in the ganglion on leaving nerve 5 before turning toward the midline and branching in an area anterior to that occupied by the branches of the posterior tarsal sensory neuron (Fig. 3B). Similarly, axons from a tactile hair located on the posterior surface of the femur (Fig. 3E) projected around the posterior edge of the neuropil before turning anteriorly and branching, whereas the axon from a tactile hair on the anterior femur (Fig. 3F) took a more direct path across the neuropil before branching anterior to the arbor of the neuron from the tactile hair on the posterior femur. Thus the position of tactile hairs on the anterior or posterior surface of the leg is also reflected by the anterior or posterior central projection of their sensory neurons in the mesothoracic ganglion (Fig. 3C,G).

Projections of sensory neurons innervating basiconic sensilla

The greatest spatial separation between central projections of sensory neurons from tactile hairs was for neurons from hairs on the distal three leg segments (the femur, tibia and tarsus). We therefore chose basiconic sensilla on the dorsal surface of these three distal leg segments to compare their central projections with those from tactile hairs along the proximo-distal axis of the leg.

Figure 4 illustrates two representative examples of sensory projections from basiconic sensilla from each of these three regions of the leg (femur, n = 46; tibia, n = 34; tarsus n = 10). In the examples of projections from basiconic sensilla on the proximal dorsal femur, four (Fig. 4Ai) and two (Fig. 4Aii) axons respectively entered the ganglion via nerve 5 and ran anteriorly and centrally, terminating in an area of ventral neuropil midway between the anterior and posterior borders of neuropil, just lateral to a line drawn between the medial edges of the connectives. Axons from two basiconic sensilla on the proximal dorsal tibia (Fig. 4Bi,ii) took different routes to their destination (see also Fig. 6), but both gave rise to numerous small branches in the same region of neuropil, lateral to the central projections from the femoral basiconic sensilla. The central projections of sensory neurons from basiconic sensilla on the dorsal tarsus projected more laterally still (Fig. 4Ci,ii). Thus, the positions of basiconic sensilla on the proximo-distal leg axis are represented by the positions of the arborizations of their sensory neurons along a medio-lateral axis in the ganglion (Fig. 4E), an organisation similar to that of the sensory neurons from tactile hairs. This mapping can be clearly seen in a preparation in which sensory neurons from basiconic sensilla on both the femur and the tarsus were stained in the same preparation (Fig. 4D). Two clear projection sites were evident, one that overlaps with the area where femoral basiconic sensory neurons project (Fig. 4A), and another that overlaps with the area to which tarsal basiconic sensory neurons project (Fig. 4C).

Parallel overlapping maps from tactile hairs and basiconic sensilla

The central mapping of sensory neurons from basiconic sensilla along a medio-lateral axis depending on their position on the leg is remarkably similar to the proximo-distal mapping of sensory neurons from tactile hairs (Figs. 4, 5). The total region occupied by the arborizations of the representative basiconic sensilla from the femur (Fig. 4Ai), tibia (Fig. 4Bi), and tarsus (Fig. 4Ci) is illustrated by light stippling in the examples drawn. The darker stippling indicates the areas occupied by the arbors of sensory neurons from tactile hairs in comparable spatial locations on the leg. It is clear that the sensory neurons from the basiconic sensillum on the proximal dorsal femur overlap with the central projections of the femoral hair afferent (Figs. 2D, 4Ai). Likewise, the central projection from the basiconic sensillum on the proximal dorsal tibia overlap with the central projections of a nearby tactile hair afferent (Figs. 2E, 4Bi), and those from the basiconic sensillum on the dorsal tarsus overlap with the central projections of a tactile hair afferent on the dorsal tarsus (Figs. 2F, 4Ci).

This parallel overlapping mapping of sensory neurons from basiconic sensilla and tactile hairs was consistent for all receptors analysed. The average areas covered by the arborizations of basiconic and trichoid sensilla sensory

Fig. 3. (Overleaf) Mapping of sensory neurons from tactile hairs according to the antero-posterior leg axis. **A:** The central projections from a tactile hair on the posterior surface of the tarsus of the middle leg. **B:** The central projection of a sensory neuron from a hair on the anterior tarsus. The stippled area indicates the area occupied by the branches of the sensory neuron shown in A. **C:** Superimposing the drawings of both sensory neurons shows the more anterior projections of the anteriorly located tactile hair. **D:** The locations of the tactile hairs on the mesothoracic tarsus. **E:** The central projections of the sensory neuron from a tactile hair on the posterior femur. **F:** Central projections from a tactile hair on the anterior surface of the femur. The stippled area indicates the region occupied by the branches of the tactile hair afferent depicted in E. **G:** Superimposed drawings of the arborizations of sensory neurons from the sensilla on the anterior and posterior femur. **H:** Locations of the tactile hairs on the mesothoracic femur.

Fig. 4. Mapping of the central projections of sensory neurons from basiconic sensilla along the proximo-distal leg axis. **Ai, ii:** Two examples of the central projections from basiconic sensilla located on the proximo-dorsal femur. **Bi, ii:** Projections of sensory neurons from basiconic sensilla situated on the proximo-dorsal tibia. **Ci, ii:** Central projections from basiconic sensilla located on the dorsal tarsus. On the ganglia drawn, the light stippling represents the area occupied by the sensory neurons from all the basiconic sensilla. The darker stippling indicates the projection areas of tactile hair afferents from similar proximodistal locations of the middle leg taken from Figure 2. Note the close correlation between the branching areas of sensory neurons from both classes of receptor. **D:** Staining a basiconic sensillum on the femur and another on the tarsus in one animal shows a clear separation in projection areas within the ventral neuropil. **E:** Superimposing the drawings of sensory neurons from different areas on the leg (**F**) shows the central projection from the basiconic sensilla map according to the spatial position of their corresponding receptor on the proximo-distal axis of the leg.

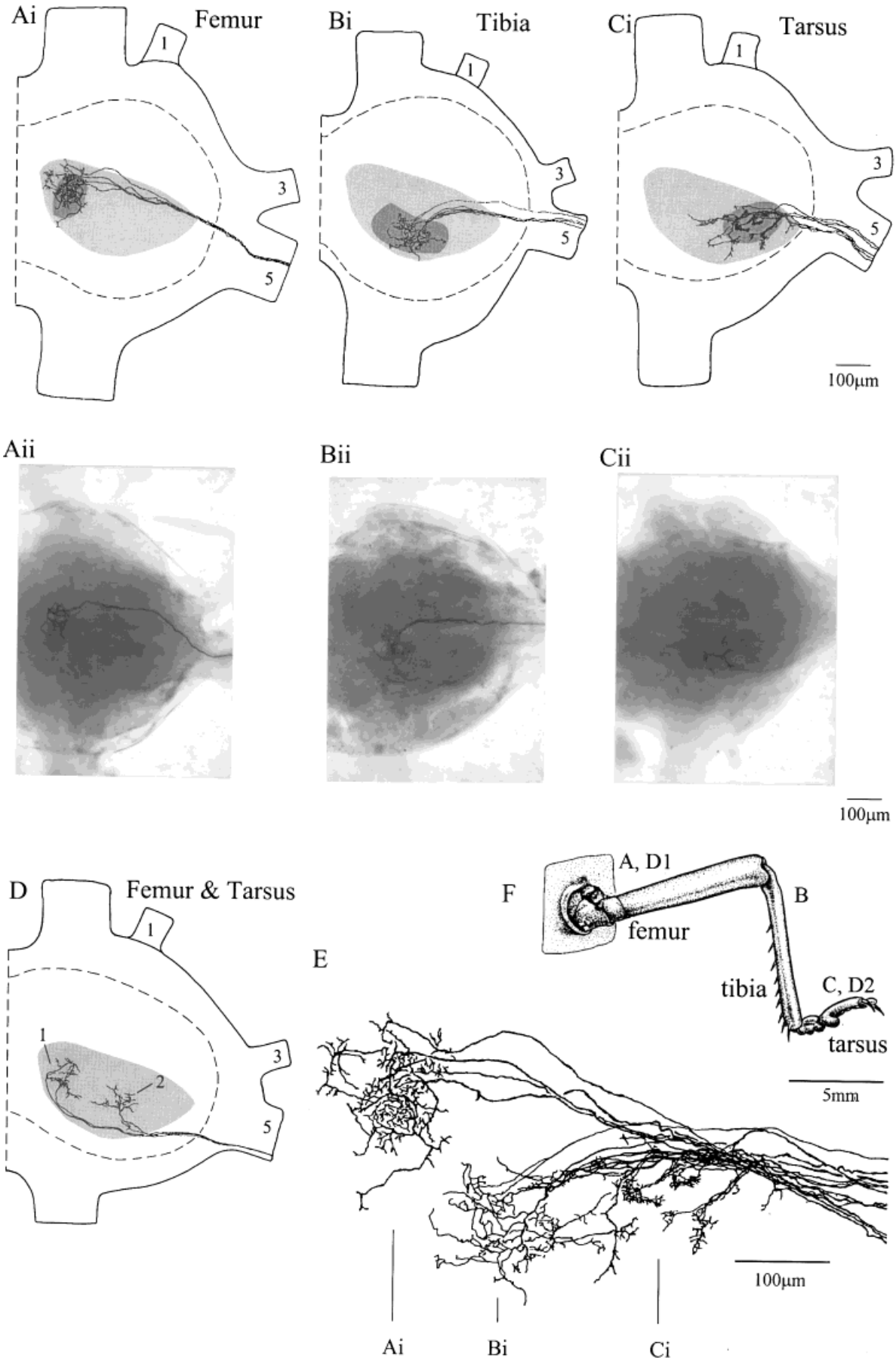
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Figure 4

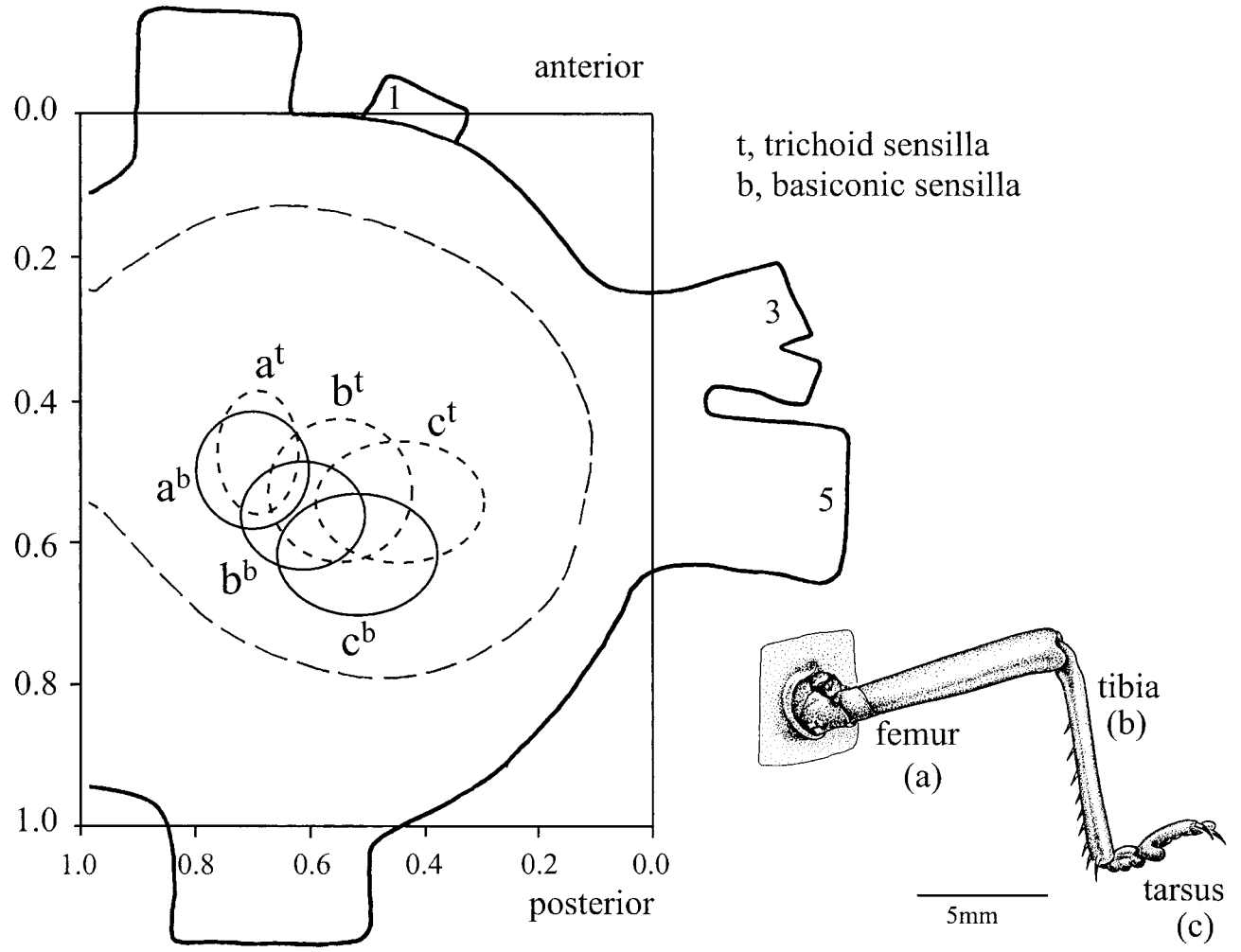

Fig. 5. Average positions of the most anterior, posterior, medial, and lateral extents of the arborizations from basiconic (solid lines) and trichoid sensilla (dotted lines) from the femur (**a**), tibia (**b**), and tarsus (**c**), as shown by ellipses connecting each of the four points. Each position was calculated as the ratio of the distance from the anterior or lateral edges of the ganglion (0 on the axes of the figure) to the extremities of the projections relative to the total length or maximum width of the hemiganglion. Average positions were calculated from 46 femoral, 34 tibial, and 10 tarsal basiconic sensilla and 10 femoral, 9 tibial, and 9 tarsal trichoid sensilla. t, trichoid sensilla; b, basiconic sensilla.

neurons were quantified by computing the mean most anterior, posterior, medial, and lateral extents of the projections from all successful backfills, in which the full extent of arborization was clear, from sensilla on the femur, tibia, and tarsus as a ratio of ganglion width and length. This analysis (Fig. 5) strongly supports the organisational patterns deduced from comparing representative backfills. All the sensory neurons from both tactile hairs and basiconic sensilla project in an ordered fashion within the mesothoracic ganglion, and this ordering is somatopic, reflecting the position of the receptor on the long axis of the leg. There is, however, some overlap between the average arbors from sensilla on adjacent parts of the leg, with the standard deviations of the midpoints of each arbor ranging from 0.06 to 0.1 for both sensilla types in both directions.

This overlap is also apparent in the superimposed drawings of the sensory projections from tactile hairs (Fig. 2G)

Fig. 6. Axonal projections of basiconic sensilla. Sensory afferents from basiconic sensilla travel across the ganglion and arborize in a number of different ways, but there is no consistent observable spatial separation of neurons into different regions consistent with differences in modality. Projections from single basiconic sensilla on the dorsal tibia. **Ai, ii, iii:** Three examples of projections in which the axons run in a narrow bundle anterior of nerve 5 before turning toward the posterior and arborizing in two distinct zones connected by a narrow waist. **Bi, ii, iii:** Three examples of sensory projections in which the axons travelled in a diffuse bundle across the ventral neuropil before arborizing in a variety of forms. **Ci, ii, iii:** Three examples of sensory projections from single sensilla in which some axons ran around the posterior edge of the neuropil and others travelled more directly to their arborization region. For each drawing anterior is to the top, and the midline of the ganglion is to the left. The photographs show two further examples of sensory projections in which (**D**) the neurons travel together in a narrow bundle or (**E**) take divergent routes to their arborization region.

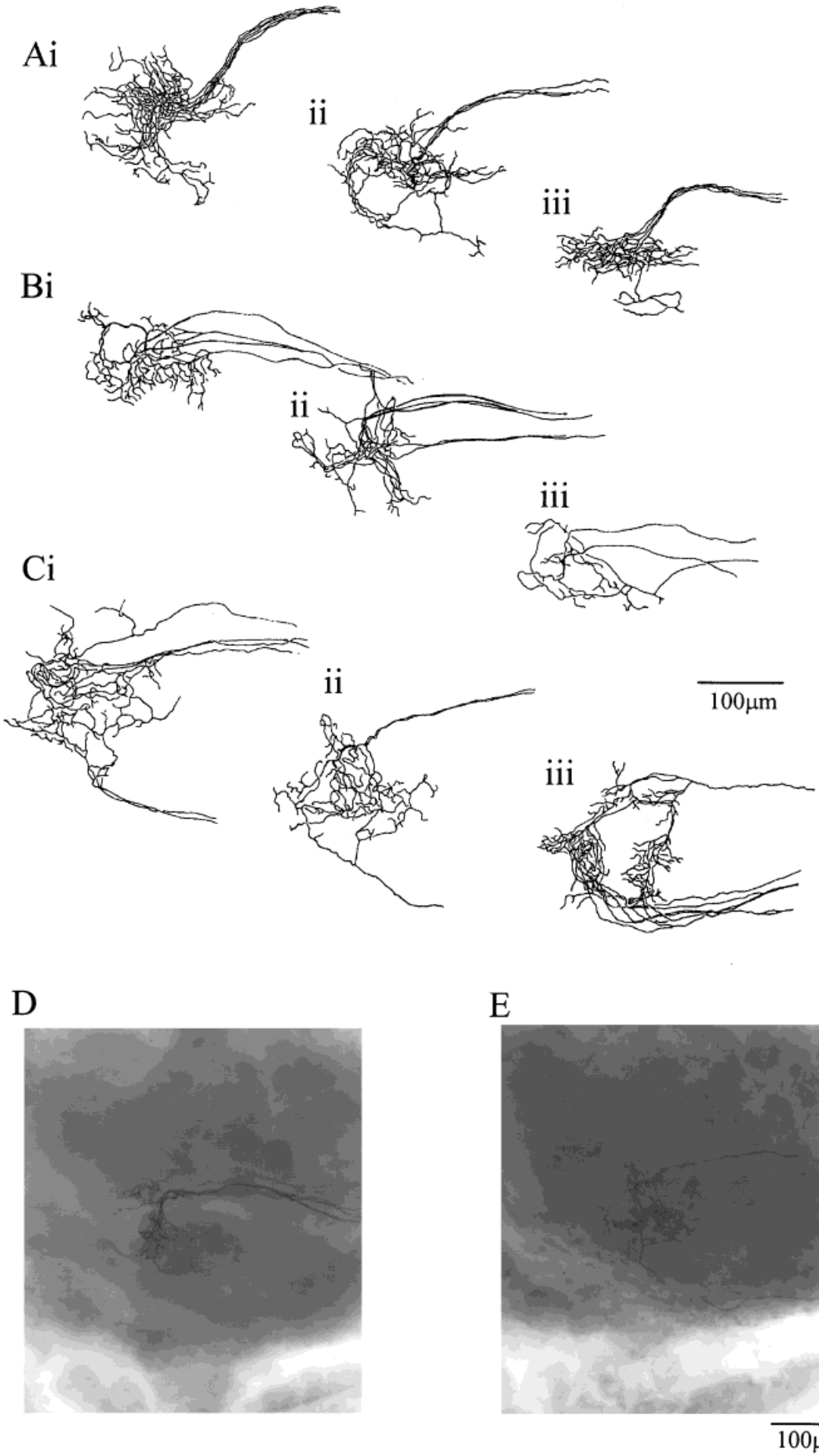

Figure 6

and basiconic sensilla (Fig. 4E) from over the entire axis of the leg. Nevertheless, correlation analyses comparing the distance of the midpoints of the arborizations of sensory neurons from the midline of the ganglion to the positions of the sensilla on the legs that they innervate were significant for both basiconic (Pearson correlation -0.662, $P < 0.0009$, n = 74) and trichoid sensilla (Pearson correlation -0.859, $P < 0.0009$, n = 29). It is possible that because only a single location was used in most preparations, for any individual animal the ordering of sensory neurons on adjacent parts of the leg is such that the degree of overlap is minimised. The method used here precludes any analysis of how the abors of one neuron may relate to others within the same ganglion. There is also a close similarity in the positions of the arbors of tactile hairs and basiconic sensilla from comparable locations on the leg. The arbors of basiconic sensilla are, on average, a little more medial and posterior relative to those from tactile hairs, but there is a considerable overlap in the branches from both sensilla types. Taking into account the variability of positions exhibited by individual sensilla, it appears that not only are the arbors of tactile hairs and basiconic sensilla contiguous, but to a large extent they are also intercalated.

Organisation of sensory afferents from basiconic sensilla

The total area occupied by the arborizations of all the sensory neurons from individual basiconic sensilla was similar to the area occupied by those of the single neurons from tactile hairs (Fig. 5). Moreover, the total arborization area of the projections from individual basiconic sensilla was not significantly correlated with the number of axons staining in nerve 5 (area calculated by multiplying arborization length by width ratios as described above; Spearman's coefficient = 0.198, $P > 0.05$, n = 74).

The projection patterns of 19 basiconic sensilla on the dorsal tibia were analysed in detail to determine whether there were any clear differences in the projection areas of the different neurons that might indicate separate destinations for neurons with different modalities or sensitivities (Fig. 6). The numbers of sensory neurons within the basiconic sensilla on the leg have not been systematically investigated, although five neurons, one of which is mechanosensory, have been reported for some leg sensilla in locusts (Chapman, 1982). Conversely, numbers of sensory neurons within palp-dome gustatory sensilla are known to be variable (Blaney et al., 1971).

Therefore, the variability in the number of axons staining in the mesothoracic ganglion may reflect genuine differences in the sensory neuron complement of basiconic sensilla as well as experimental artefacts. Over 83% of successful stains from basiconic sensilla on the tibia consisted of three or more axons entering the ganglion, with 44% of stains consisting of five or six neurons, the maximum number stained. As with the sensory neurons from tactile hairs, axons from basiconic sensilla entering the ganglion took a number of routes to their destination. In 7 of the 19 projections analysed, all the stained axons travelled in a narrow bundle and took a path anterior and medial of nerve 5 before curving back and beginning to arborize (Fig. 6Ai–iii,D). In other projections, the axons travelled in a more widely spaced diffuse bundle across the ganglion (Fig. 6Bi–iii), with some axons taking anterior paths and others travelling more directly to their destination. In a further five preparations, the axon paths through the ganglion were widely divergent, with at least one axon travelling around the posterior edge of the neuropil before turning toward the anterior and branching (Fig. 6Ci–iii, E). There were no instances of stains consisting of more than one axon that exclusively took this posterior route. The variety of paths would seem to indicate genuine differences between sensilla rather a variety of partial stains, as there was no difference in the mean number of axons in stains where the neurons ran directly to their destination compared with stains where axons took both anterior and posterior paths (Mann-Whitney test, $Z = -0.97$, $P = 0.945$, n = 29, range in axon numbers 1–6 in both types).

Furthermore, there was no indication of a consistent numerical differentiation between the number of neurons taking anterior and posterior paths that might suggest a modality-linked difference in route (Fig. 6C). There was also no clear indication of the consistent presence of axons with different diameters that could correlate with the presence of a single mechanosensory and several chemosensory neurons, such as have been reported for the sensory projections from bimodal gustatory sensilla of Diptera (Edgecomb and Murdoch, 1992).

The arborizations of stains from basiconic sensilla on the tibia were further examined to determine whether there were any readily apparent spatial subdivisions between the branching patterns of different neurons within the "tibial region" that could be related to differences in modality or chemical sensitivity. A common arborization pattern, particularly associated with stains in which the axons travelled closely together, was for the neurons to arborize extensively in two separate regions linked by a narrow connection (Fig. 6Ai). Although there was one instance in which the distalmost arbor was clearly composed of branches from a single neuron (Fig. 6Aiii), in all other stains both regions consisted of branches from two or more neurons (Fig. 6Ai,ii,D). In preparations in which axons approached their arborization area from different directions, some of the neurons commonly bifurcated and travelled some distance further before giving rise to their main mass of branching, resulting in some cases in spatially separate branching regions (Fig. 6Cii–iii). Each region, however, received branches from more than one neuron; there were no instances of exclusive regions composed of arbors from single neurons only. Therefore, although there are a number of different branching patterns, which may give rise to spatially separate areas of arborization, there is no observable evidence that these zones are exclusively composed of branches from individual neurons. Consequently there is no evidence to support a modality- or sensitivity-dependent spatial separation of neurons across the ganglion.

Dorso-ventral projections of sensory neurons from basiconic sensilla

Several ganglia containing stains of either basiconic or trichoid sensilla afferents were drawn in thick transverse sections to determine whether there could be any dorsoventral partitioning of sensory afferents consistent with modality. Axons from trichoid sensilla on the middle leg run along the ventral edge of the neuropil before turning in a slightly dorsal direction to arborize in the lateral ventral association centre (lVAC) (Fig. 7Aii). This organisation has already been described for the projections of

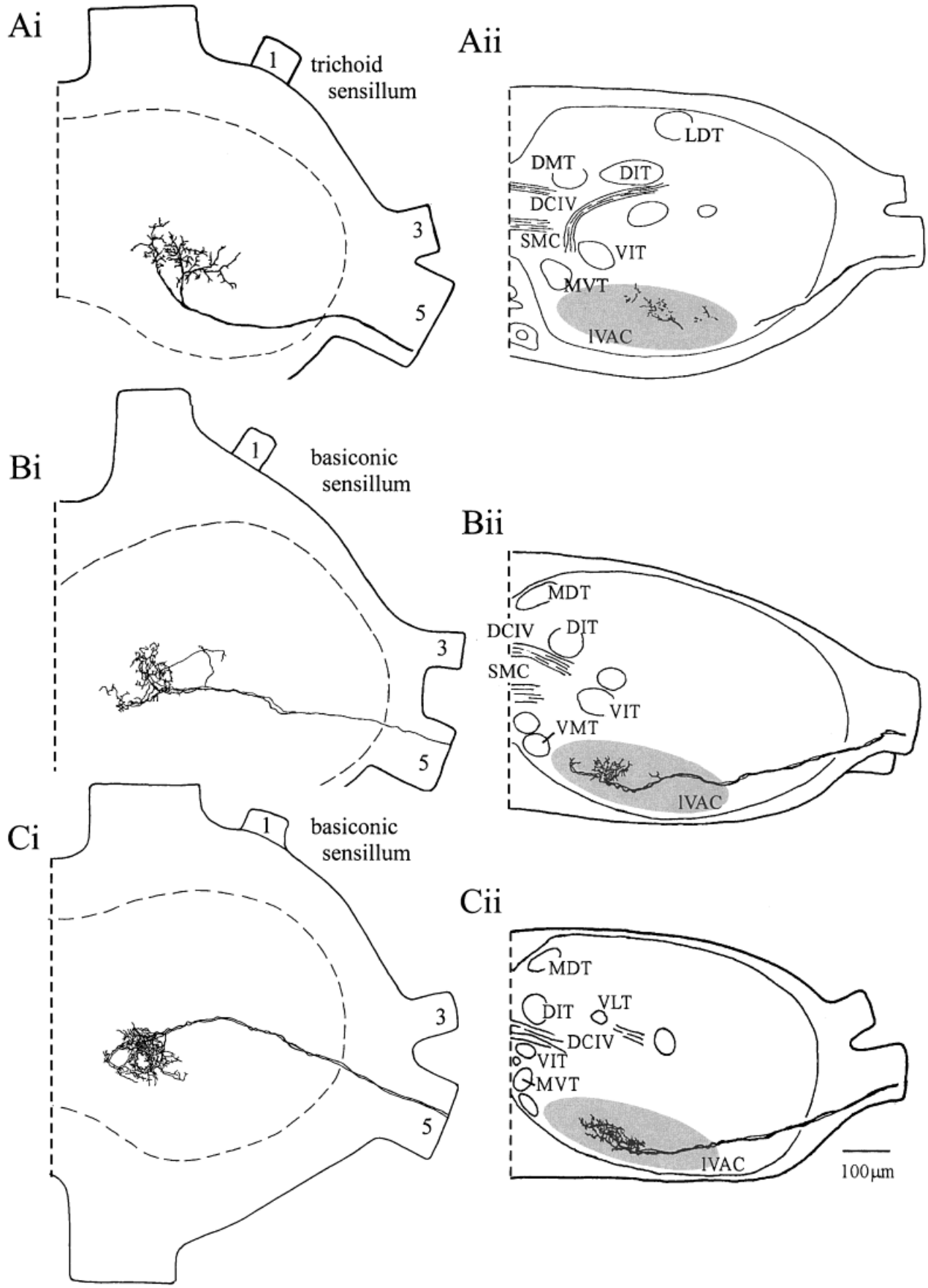

Fig. 7. Sensory afferents from both trichoid and basiconic sensilla project to the same part of the ventral association centre. **A:** Reconstruction of the sensory neuron from a trichoid sensillum located on the dorsal femur shown in wholemount (**i**) and in section (**ii**). **B:** Sensory neurons from a basiconic sensillum on the dorsal tibia, shown in wholemount (**i**) and in section (**ii**). **C:** Sensory afferents from a basiconic sensillum on the dorsal femur, shown in wholemount (**i**) and in section (**ii**). There was no apparent dorso-ventral separation of basiconic sensilla afferents consistent with any putative differences in modality. The lateral ventral association centre (lVAC) is shown in grey.

sensory neurons innervating both hind and middle leg tactile hairs in the meta- and mesothoracic ganglia (Newland, 1991; Mücke and Lakes-Harlan, 1995). Two features of the central projections of basiconic sensilla sensory neurons were apparent in the transverse sections (Fig. 7B,Cii). First, the arborizations of all the sensory neurons occur within the same region of the lVAC as that occupied by tactile hairs afferents from similar locations on the leg. Second, the branches of all the sensory neurons were intermingled. There was no clearly observable separation between different neurons consistent with the existence of spatially separate neuropil regions for processing the different modalities. All basiconic sensillum afferents branched within a restricted dorso-ventral region within the lVAC regardless of the route the axons took across the ganglion to reach their arborization site.

DISCUSSION

We have demonstrated that the sensory neurons from both trichoid and basiconic sensilla on the middle legs of locusts are organized into parallel and largely overlapping somatosensory maps within the mesothoracic ganglion and that the position of the sensilla on the leg is the major correlate of the destination of its sensory projection. Furthermore, because all the sensory neurons from individual basiconic sensilla terminate within the same region of neuropil, it appears that not only are all mechanosensory neurons arranged somatotopically, but that the gustatory neurons from these sensilla also follow a closely similar organisation.

The extent of the somatosensory map of tactile hair afferents on the middle leg and mesothorax revealed by this study extends the scope of earlier work by Mücke and Lakes-Harlan (1995) in which only the distalmost three leg segments were analysed and that was unable to demonstrate the presence of any anterior-posterior organisation. It has now been established that there is a complete three-dimensional mapping of tactile hair location on the middle leg encompassing proximo-distal, anterior-posterior, and dorso-ventral axes, all of which are faithfully represented in the mesothoracic ganglion. In this respect the arrangement of tactile hair afferents from the middle leg closely resembles that of tactile hair afferents on the hind leg as described by Newland (1991) and follows the well-established pattern for leg bristle afferent sensory projections described in other insects (e.g., Johnson and Murphey, 1985; Murphey et al., 1989b; Pflüger et al., 1981). Establishing this framework for the sensory projections of tactile hair afferents then allowed us to compare this map with the unknown projections from the bimodal basiconic sensilla, which we have shown to be organisationally and spatially similar to that of the tactile hairs. This has implications for the organisation of both exteroceptive and contact-chemosensory processing within the thoracic ganglia.

Exteroceptive organisation and processing

Previous physiological studies had already provided some evidence to support a close spatial association between the processes of mechanosensory neurons from both trichoid and basiconic sensilla. Spiking local interneurons, which are responsible for much of the initial processing of sensory signals in the thoracic local circuits of insects (Burrows and Siegler, 1982, 1984), receive monosynaptic inputs from the mechanosensory neurons innervating both basiconic and trichoid sensilla (Siegler and Burrows, 1983; Newland and Burrows, 1994; Burrows and Newland, 1994). These local interneurons have specific receptive fields, determined by the pattern of sensory inputs they receive from mechanosensory afferents on different parts of the leg. Both types of sensilla are freely intermingled over the surface of the leg, and the receptive field of any given spiking local interneuron is similar for both classes of mechanosensory afferent. The input branches of spiking local interneurons are largely restricted to the same regions of neuropil as the tactile hair somatosensory map (Newland, 1991), and their receptive field properties are strongly correlated with the pattern and degree of overlap their branches make with the tactile hair afferents (Burrows and Newland, 1993). Since both tactile hair afferents and their target postsynaptic neurons follow a somatotopic organisation and these same interneurons also receive mechanosensory inputs from basiconic sensilla, then a similar somatotopic projection pattern of at least the mechanosensory afferents from basiconic sensilla would be the most parsimonious arrangement possible.

Chemosensory afferent organisation

The more surprising finding of this study was that all the sensory neurons from basiconic sensilla, both mechano- and chemosensory, projected to the same regions of the lVAC as determined by the location of the sensilla on the leg. We could find no anatomical evidence to support either a spatial partitioning, or any other differences in sensory neuron structure, such as neurites with different diameters or branching patterns, consistent with a differentiation of the two modalities of neuron.

The few studies that have examined the responses of basiconic sensilla on the legs of insects suggest that there is no systematic variation in the chemosensory responses of the sensory neurons of basiconic sensilla from different locations on the leg (Blaney and Winstanley, 1980; White and Chapman, 1990). It is therefore likely that the organization we describe is somatotopic and does not arise coincidentally from differences in chemosensory specificity of receptors on different locations of the leg. Anatomical techniques alone, however, cannot rule out specificity of synaptic connections within apparently similar fields of branches.

Much less is known about the initial stages of chemosensory integration by the local circuits in the thoracic ganglia compared with mechanosensory processing, but there is some new evidence that at least some chemical stimuli may be processed by the same neurons as exteroceptive stimuli. First, the mechanosensory receptive field of spiking local interneurons largely coincides with the chemosensory receptive field determined by targeting acidic vapours to different parts of the leg (Newland, 1999). Second, for any particular interneuron, the polarity of both mechano- and chemosensory inputs is always the same (excitatory/inhibitory) from receptors on specific regions of the leg (Newland, 1999).

It has also been shown that just as spiking local interneurons receive monosynaptic mechanosensory inputs (Burrows, 1992), they also appear to receive monosynaptic inputs from chemosensory neurons from the same bimodal basiconic sensilla (Newland, 1999). The leg withdrawal reflex performed by locusts on stimulation with acidic

vapours (Newland, 1998) or droplets containing sufficient concentrations of other chemicals (Rogers and Newland, 2000) is quite similar to the withdrawal reflex following tactile stimulation (Pflüger, 1980; Siegler and Burrows, 1986) and suggests there may be a similar underlying neural organisation. It is still not clear, from physiological data, however, whether all chemosensory stimuli are processed by these same interneurons or whether only certain classes of chemosensory neuron synapse onto the midline spiking local interneuron population. The somatotopic projections of all basiconic sensilla afferents to the same region would tend to support the former proposition.

Comparison with other insects

The data shown here contrast with the data presented in other studies examining the sensory projections of bimodal sensilla on the legs or mouthparts of other insects. Few systematic analyses have been performed on other insects, and such data as are available are sometimes contradictory. Murphey et al. (1989a) suggested that in *Drosophila*, afferents from tactile bristles formed a clear somatotopic map of the proximo-distal leg axis in the mesothoracic ganglion. In a separate analysis of the central projections of contact chemoreceptors (bimodal sensilla) on the tarsi of the foreleg, Murphey et al. (1989b) suggested that there was a spatial segregation of sensory neurons with different modalities. This study did not address the problem of the proximo-distal mapping of sensory neurons from contact chemoreceptors over the entire leg. It did show, however, that that the putative mechanosensory neuron from gustatory sensilla projected to the same area as tactile bristle afferents from the tarsus (Murphey et al., 1989a). It has commonly been found in studies of the sensory projections of bimodal sensilla in Diptera that one of the afferents is of larger diameter than the others (Yetman and Pollack 1986; Murphey et al., 1989b; Edgecomb and Murdock, 1992), and it was this afferent that projected to the same region as tactile bristle afferents.

It is possible that chemosensory projection patterns are organised in a different way in the Orthoptera and Diptera, as there is clear evidence of a spatial separation of presumed mechanosensory and chemosensory neurons on the labella (mouthparts) of flies. There are 11 identifiable long contact chemoreceptors on the labellum of the blowfly, each innervated by a single mechanosensitive afferent, and four chemosensory afferents, all with different chemical sensitivities (Dethier, 1976). The central projections of the thicker, presumed mechanosensitive, afferents from these contact chemoreceptors formed a discontinuous map that reflected the spatial position of the sensillum on the labellum (Yetman and Pollack, 1986; Edgecomb and Murdoch, 1992). The remaining, presumably chemosensory, neurons projected predominantly to a more ventral and medial region of neuropil, although some sent processes to the brain. Sensory projections from contact chemoreceptors on the mouthparts of locusts, which are involved in making detailed assessments of food quality, may have a different neural organisation than is found in the thoracic ganglia and may more closely resemble the organisation seen in the Diptera.

Implications for chemosensory processing by the thoracic ganglia of locusts

The repeated representation of chemosensory neurons with similar sensitivities in different spatial locations in a purely somatotopic map argues that gustatory processing in the thoracic ganglia is organised in a very different manner from olfaction in locusts. There is increasing evidence that the antennal lobes, the primary olfactory neuropil in insects, are arranged in an odotopic manner (Vickers et al., 1998). Particular functional classes of olfactory receptor neurons with similar sensitivities project to the same compartment, or glomerulus, in the antennal lobe regardless of the position of the olfactory receptor on the antenna. Indeed, in some insects, the antennal lobes also receive sensory projections from subsidiary olfactory organs located elsewhere on the head, such as the maxillary palps of Diptera (de Bruyne et al., 1999). Different odours are represented by the patterns of activity across the total population of glomeruli.

In contrast to the convergence of all olfactory receptor neurons onto the same integrative region, the initial processing of contact-chemosensory signals by the thoracic ganglia appears highly redundant. Chemosensory stimulation of basiconic sensilla on the leg may evoke local reflex movements, which are always similar regardless of the chemical used, even if the chemical is a nutrient or other phagostimulant (Rogers and Newland, 2000). Chemical identity and concentration strongly affect the probability of this response occurring, and blends of different chemicals may increase or even decrease the likelihood of response compared with the constituents applied individually. Clearly, local circuits controlling leg movements use information about chemical identity and concentration to determine whether to perform a leg withdrawal response, but it is perhaps unlikely that local circuits in the thorax encode individual chemical qualities; they may only use a generic index of aversiveness in reaching a decision. It is then possible that both mechanosensory and chemosensory information may be combined in the same local circuits at this level.

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