Strand receptors with central cell bodies in the proximal leg joints of orthopterous insects

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Summary. In various orthopterous insects backfilling of leg nerve 3B regularly stained, in the thoracic ganglia, small cell bodies that resemble those of central sensory neurons reported in the locust (Bräunig and Hustert 1980). Centrifugal cobalt infusion of this nerve revealed the end organs of those neurons in the periphery. In all species investigated one strand receptor is associated with the trochantin, while two others are situated in the coxa. In addition to these sense organs, the coxa contains a multipolar stretch receptor which spans the coxo-trochanteral joint. The absence of chordotonal organs is discussed with reference to earlier work in this field.

Key words: Orthoptera – Leg joint – Proprioceptor – Cobalt staining

In addition to one main leg nerve, branches of other lateral nerves contribute to the afferent and efferent innervation of the leg in orthoptera. In the cockroach and in the locust most of the sense organs of the proximal leg joints for instance send their axons via the second branch of the third lateral nerve root (N3B) towards the thoracic ganglia (Nijenhuis and Dresden 1952, 1955; Bräunig et al. 1981).

In his backfills of the locust metathoracic N3B Burrows (1975) noticed a group of 12–15 small-diameter cell bodies in the anterior ipsilateral region of the ganglion. Later about one half of these neurons were shown to be sensory and to innervate a small connective-tissue strand in the thoracocoxal joint of the hind leg (Bräunig and Hustert 1980). It seemed likely that the remaining cells in the group might innervate coxal sense organs of the same type (strand receptor) as that already described in more detailed studies of the sensory supply of the proximal joints of locust legs (Bräunig et al. 1981; Hustert et al. 1981; Pflüger et al. 1981).

In the locust, strand receptors seem to be the only internal proprioceptors in the coxa whereas Nijenhuis and Dresden (1952) described three chordotonal organs in

the cockroach coxa. This apparent discrepancy in the sensory supply in insects of the same order seemed worth reinvestigating. Other orthopteran species were included to extend the comparison. Additionally, experimental procedures (see below) were chosen to test our hypothesis (Hustert et al. 1981) that once the specific central sensory projections of a particular body region are known, this information can be used to interpret backfills of major nerves innervating the corresponding peripheral areas.

Materials and methods

Orthopteran species from five different families were used: *Locusta migratoria* (Acridiidae), *Acheta domesticus* (Grillidae), *Extatosoma tiaratum* (Phasmidae) and *Periplaneta americana* (Blattidae) were taken from laboratory cultures, while a few specimens of *Gryllotalpa gryllotalpa* (Grillidae) and *Decticus verrucivorous* (Tettigoniidae) were collected around Konstanz.

The insects, immobilized by chilling, were pinned down ventral side up, and the meso- or metathoracic N 3 B exposed by removal of the basisternum. The nerves were cut and the proximal and/or distal stumps were placed in small vaseline beakers containing 0.5–3 % solutions of cobaltous chloride. Low concentrations (0.5–1 %) yielded best results with both centripetal and centrifugal axonal infusion of cockroach nerves, whereas for all other species higher concentrations were required.

After incubation periods of 14–20 h at 6° C ganglia and/or proximal parts of the legs were removed and subjected to standard precipitation, fixation and clearing procedures (Pitman et al. 1972). Ganglia were studied as unintensified whole mounts and sense organs were located in whole or partly dissected legs. Specific backfills of single sense organs, as well as weakly stained sensory structures, were later silver-intensified (Bacon and Altman 1977).

Data were evaluated in three successive steps:

i) Preliminary interpretation of unintensified backfills of whole N 3 B according to the data available for the locust (Bräunig et al. 1981; Hustert et al. 1981; Pflüger et al. 1981).

ii) Survey of the periphery for different types of sense organs predicted from the overall central projection pattern.

iii) Specific centripetal fills from single sense organs as confirmation of the assumption made under i).

Results

Ganglionic backfills of N 3 B in the four principal species investigated are remarkably consistent with regard to the position of neuron somata and primary neurites, and the course followed by sensory-fiber populations (Fig. 1, top row). Generally, staining N 3 B apart from motoneurons reveals a prominent cluster of small cells in the anterior ipsilateral region of the ganglia. Their position and the typical course of their primary neurites and branches (compare Bräunig and Hustert 1980) predicts the presence of sense organs of the strand-receptor type in the proximal leg joints of all four species shown.

These organs were indeed found in homologous positions as outlined in the bottom row of Figure 1. They invariably consist of a connective tissue strand, which is innervated but lacks any cell bodies; rather, the receptor neuron fibers branch extensively after contacting the strand (an example is shown in Fig. 4A).

The first strand is located in the ventral (medial in *Periplaneta*) part of the trochantin (tnSR), between the anterior and posterior rims of this sclerite. It is only in the locust, that the recetor strand connects the anterior ventral edge of the episternum with the trochantin, which in this species is reduced to a tiny piece of hard cuticle. Two other receptors were found in the coxa, both of which insert
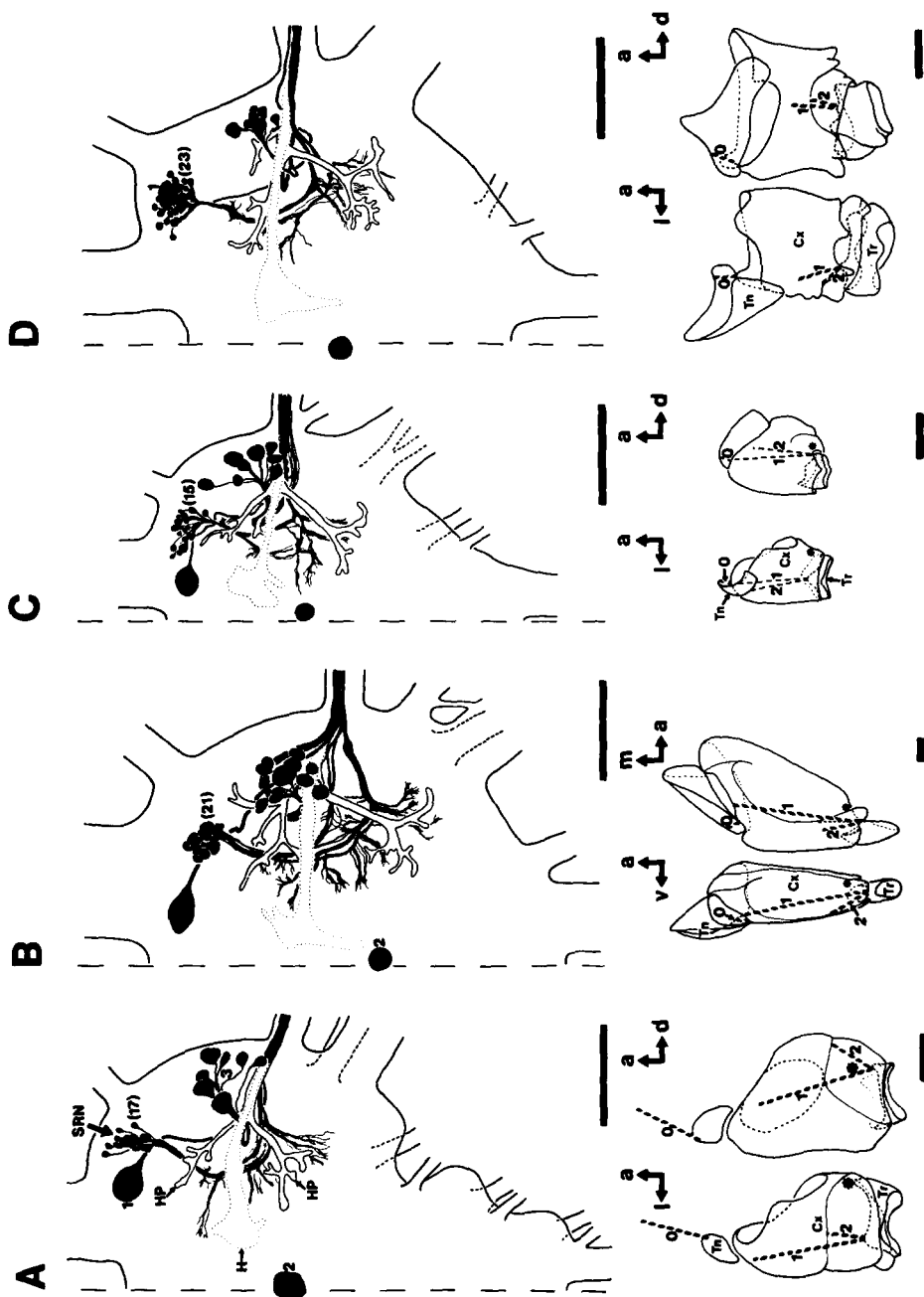
Fig. 1A–D. Central and peripheral features of metathoracic nerve 3B of four orthopteran species: *Locusta* (**A**), *Periplaneta* (**B**), *Acheta* (**C**) and *Extatosoma* (**D**). *Upper row:* Ventral aspect of unintensified backfills (left hemiganglion, anterior to the top), dorsal and lateral projections have been omitted for better orientation. The primary neurite of neuron 2 cannot be traced in unintensified material. Sensory fiber tracts that derive from hairplates (*HP*) in the locust are indicated by solid lines, those of single hairs (*H*) by broken lines. For further details, see discussion. Scale: 250 µm. *Lower row:* Dorsal (left) and lateral (right) aspects of left trochantin (*tn*), coxa (*cx*) and trochanter (*tr*) sketching the location of strand receptors (*brocken lines*) and multipolar stretch receptors (*asterisks*). *O* trochantinal; *1,2* coxo-trochanteral strand receptors 1 and 2, resp; *a* anterior, *d* dorsal, *l* lateral, *m* medial, *v* ventral (cockroach coxa tilted into homologous position). Scales: 1 mm
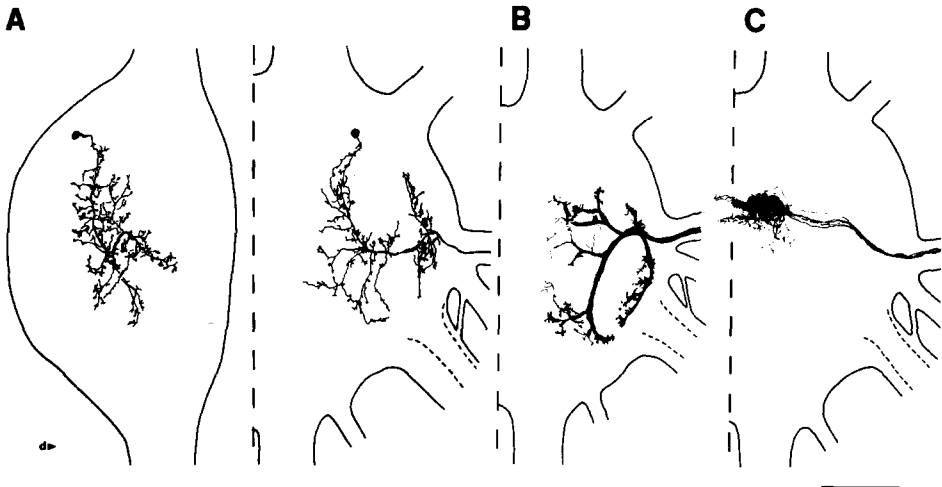
Fig. 2A–C. Camera lucida drawings of ventral and lateral (**A** only) aspects of specific central projections from different coxal sense organs of *Periplaneta* (anterior to the top). (**A**) strand receptor (cxtrSR2), (**B**) hairplate, and (**C**) single hairs; *d* dorsal. Scale: 250 μm
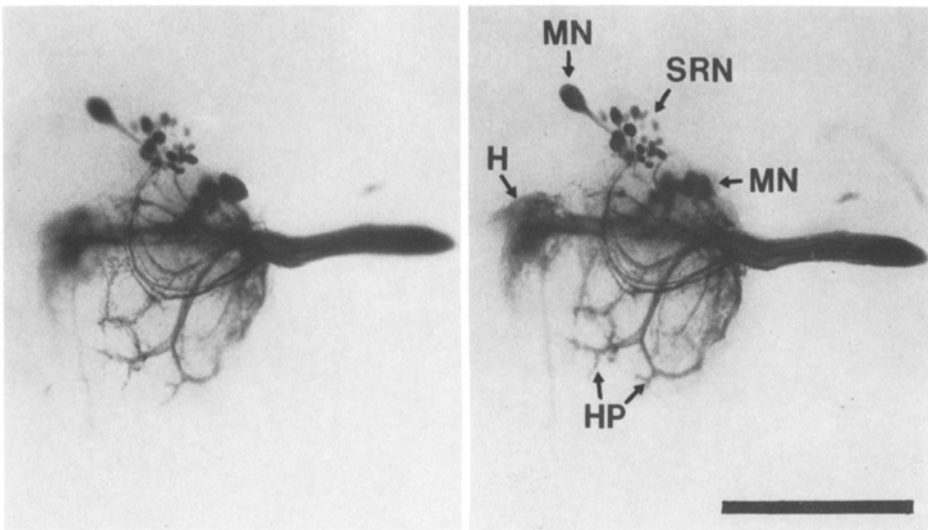

Fig. 3. Stereomicrographs of an unintensified backfill of metathoracic nerve 3B of *Periplaneta* illustrating the spatial relationship of single hair (*H*) and hairplate (*HP*) projections, strand receptor neurons (*SRN*) and motoneurons (*MN*); anterior to the top, lateral to the right, ventral view. Scale: 500 μm

distally on the apodeme of the trochanter levator muscles. Proximally, the larger one (cxtrSR1) is attached close to the anterior proximal (ventral proximal in *Periplaneta*) rim of the coxa. The second (cxtrSR2), which is shorter and thinner than the first, has its proximal insertion more dorsal than cxtrSR1, except in the cockroach where cxtrSR2 lies slightly medial ("below") to cxtrSR1. CxtrSR2 in *Decticus* and *Gryllotalpa* has not yet been found.
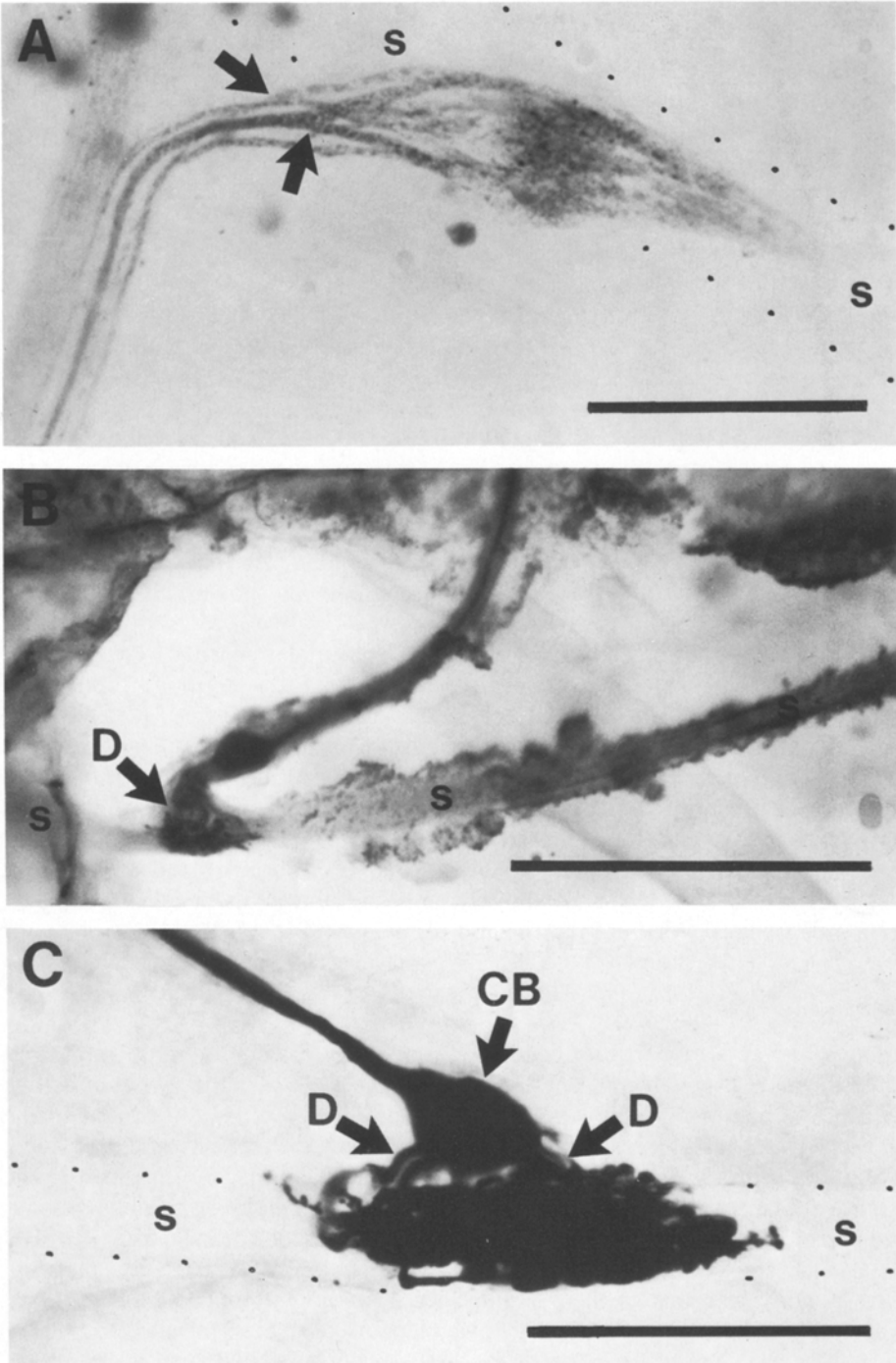
Fig. 4A–C. Photomicrographs of coxal sense organs. **A** Distal strand receptor (cxtrSR2) of *Periplaneta*. *Arrows* indicate first order branching or receptor fibers, strand (*s*) outlined by dots, distal to the right. Scale: 100 μm. **B** Multipolar receptor of *Acheta*. Note that branching of dendrites (*D*) is restricted to the distal (left) end of the strand (s). Scale: 100 μm. **C** Homologous cell in *Periplaneta* enlarged (distal to the right). At least 6 dendrites (*D*) leave the cell body (*CB*). Dots indicate strand (*s*) silhouette. Scale: 50 μm

In all species cxtrSR 1 is innervated at its proximal end, as is tnSR of the locust. Usually tnSR and cxtrSR 2 receive their innervation approximately in the middle of their strands. The smaller coxal receptor contains fewer fibers than the other two. In the cockroach, for instance, only four fibers are usually visible in the nerve supplying cxtrSR 2 compared with the 8–10 to each tnSR and cxtrSR 1 in both pterothoracic segments. This result is confirmed by specific centripetal filling of cxtrSR 2, which usually reveals four small neurons.

In one preparation just one of the cxtrSR 2 cells was stained completely (Fig. 2A). Here the morphology of a single insect sensory neuron with centrally located cell body can be seen for the first time and the differences between these receptor neurons and others become apparent.

In addition to the strand-receptor projection, Figure 2 shows specific fills of coxal hairplates (Fig. 2 B) and single hairs (Fig. 2 C). Together they illustrate the similarity between the central projections of different types of sense organs of the cockroach and those of homologous organs of the locust (Bräunig and Hustert 1980; Pflüger et al. 1981) as well as showing that the assumptions concerning the origin of the different sensory tracts visualized after filling the entire N 3 B (outlined in Fig. 1, top row) seem to be correct. The components shown in Figure 3 were labelled according to these results.

From cobalt fills like that shown in Figure 3 multipolar neurons were predicted to occur in the coxa of the locust (Hustert et al. 1981). These neurons are extremely difficult to stain and their central projections, as well as their cell bodies in the periphery (Fig. 1, bottom row), are usually visible only after intensification. So far they have only been found in crickets (Fig. 4 B), cockroaches (Fig. 4 C) and locusts (Bräunig, in preparation). These multipolar stretch receptors consist of an elastic strand which inserts on the dorsal (lateral in *Periplaneta*) coxal wall proximally and close to the posterior (dorsal in *Periplaneta*) condyle of the coxo-trochanteral joint distally. Located close to the distal insertion is one neuron, the dendrites of which form a dense meshwork around the end of the strand (Fig. 4 B and C).

Discussion

Among orthopterous insects the arrangement of major internal tracts and commissures (Pipa et al. 1959; Tyrer et al. 1979) as well as the number and relative positions of sensory and motoneuron cell bodies and sensory fiber tracts in the thoracic ganglion are very similar (Fig. 1, top row). Of the neurons labelled 1 and 2 in Figure 1 (top row), cell 1 has been identified as excitatory motoneuron of the extensor tibiae muscle in locusts (Burrows and Hoyle 1973) and phasmids (Bässler and Storrer 1980). Neuron 2 is known as the common inhibitor in locusts (Burrows 1973) and cockroaches (Pearson and Fourtner 1973). It is likely that the corresponding neurons of the other species serve similar functions. The lateral group of 7–8 cells (3 in Fig. 1, top row) contains only the motoneurons of the trochanter levator muscles, except in the cockroach, where N 3 B innervates another muscle (Nr. 183; see Carbonell 1947, and Nijenhuis and Dresden 1955) as well, which might explain the 10–13 cells in group 3 in this species.

Sensory neurons with their cell bodies located in the CNS seem to occur throughout the orthoptera in homologous positions and in comparable numbers

(Fig. 1, top row, numbers in brackets). The same is true of their peripheral end organs (Fig. 1, bottom row). One could argue that there is little evidence that all of these structures are sensory, since recordings have been made from only one of the locust organs (Bräunig and Hustert 1980). However, these organs have also been shown to be sensory in cockroaches by Becht (1958), who investigated the same coxal organs physiologically, although he did not know their real structure. He believed these receptors to be chordotonal organs, since Nijenhuis and Dresden (1952) in their detailed study of the innervation of coxal sense organs described tnSR as "coxotrochantinal", cxtrSR 1 as "proximal coxal" and cxtrSR 2 as "anterior distal coxal" chordotonal organs.

These authors also declared the multipolar stretch receptor (Fig. 4C) of the coxa to be a "posterior distal coxal" chordotonal organ. That this sense organ is clearly demonstrated to consist of a multipolar neuron and its receptor strand, and the cell bodies found in the central projections of other coxal receptors, is good evidence that they are not chordotonal organs (Fig. 2A). Nijenhuis and Dresden (1952) do not give their evidence for scolopidia, but refer only to Holste (1910) who, in their opinion, found a coxal chordotonal organ in *Dytiscus*. Holste, however, merely describes a small branch of the "nervus coxalis inferior" (which corresponds to N 3 B, see Nijenhuis and Dresden 1955) which "...is elastic and glitters like a chitinous thread" ["... elastisch ist und wie ein Chitinfaden glänzt"]. From the description of this "nerve" and its position one may reasonably conclude that Holste had found cxtrSR 1, which is of interest, since *Dytiscus* represents a different insect order.

Multipolar sensory neurons close to the coxo-trochanteral joint are reported here for three different orthopteran species. This confirms the results of Denis (1958), who found the same type of neuron in the coxae of a termite. Together with the comparative study of Lombardo (1973) on the position of two coxal hairplates (Pringle 1938) in various insect orders, these findings suggest that the sensory supply to the proximal leg segments of different insect orders might be comparable and homologous, particularly since the discrepancy mentioned at the beginning has been resolved.

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Note added in proof

A recent paper (Collin PS, Zoomorphology 98:227–231, 1981) also describes the tnSR of the cockroach metathoracic leg. The author's description of the dorso-ventral orientation of the central projections of strand receptors, however, is the exact mirror image of what was described for locusts (Bräunig and Hustert 1980) and therefore also contradicts the results of the present study. Additionally the author is convinced that the cxtrSR1 is a chordotonal organ, although in his Fig. 2 no cell bodies are clearly visible in the "pcCO" and the ones ascribed to the "cxtnCO" most certainly represent sensory cells of the coxo-trochantinal hairplate (unpublished observation in 15 specimens).