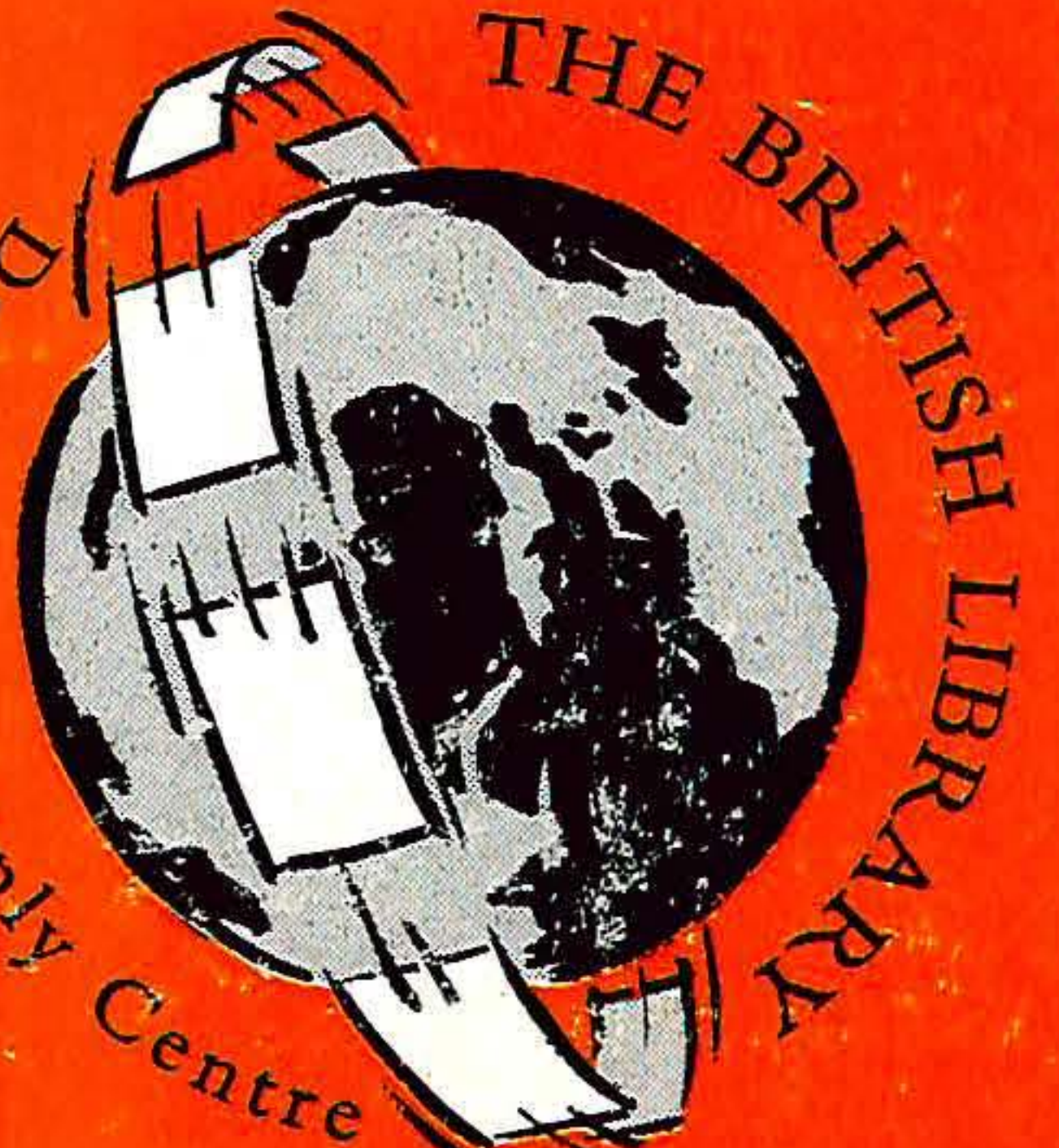


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VIBRATION RECEPTORS OF LARVAE AND OF IMAGINES IN LOCUSTS: LOCATION ON THE LEGS, CENTRAL PROJECTIONS AND PHYSIOLOGY

ALFRED GROSCH, FERNANDO CALLENDER, MARLEN PETERSEN, ANDREJ COKL & KLAUS KALMRING

ABSTRACT

The vibration receptors on the legs of grasshoppers are campaniform sensillae, subgenual receptor cells and joint chordotonal organs. In addition, numerous hair sensillae may play a role as touch or contact vibration receptors. The following features of these mechanical receptors on the six legs of adults and of 5th instar larvae of *Locusta migratoria* and of *Schistocerca gregaria* were compared: a) the number and location, b) the response characteristics and c) the central projections of the different receptors.

Most of the campaniform sensillae are arranged in groups within well defined areas on the different parts of the legs. The number and the location of these groups of campaniform sensillae on all legs are the same in 5th instar larvae as in adults, but the absolute number of campaniform sensillae within some groups in 5th instar larvae is less than in adults (Table 1). The orientation within the areas does not differ. This is true for all legs and all leg parts with exception of the tarsi.

Subgenual receptor organs are found in the blood channel of the proximal tibia of all six legs. In addition, within each leg there are located four chordotonal organs, one within the proximal femur, one near the tibio-tarsal joint and two within the tarsi. There are no differences between *Locusta migratoria* and *Schistocerca gregaria* in the number, distribution and location of vibration receptors, neither the campaniform sensillae nor the receptor cells of subgenual and chordotonal organs.

In addition, there is a large number of hair sensillae distributed on the legs of locusts. However, in *Locusta migratoria* they are far more densely packed than those in *Schistocerca gregaria* (Fig. 2).

The response characteristics, i.e. the frequency range and the suprathreshold reactivity of the different receptor cells are very similar in 5th instar larvae and in adults. There are no differences at all in the sensitivity of the responses of type I and II receptor cells, but the responses of type III receptor cells in larvae are less sensitive than in adults (Fig. 3). The projection areas of the different receptor cells in the ventral nerve cord are the same in both stages. Similar projection patterns appear in *Locusta migratoria* and *Schistocerca gregaria*.

INTRODUCTION

All orthopteran species have sensitive vibratory receptors on all six legs: the campaniform sensillae, the subgenual organs and the joint receptors. They are important for the recognition of predators, but can also be involved in conspecific communication (MARKL, -1970, 1983). The structure and especially the function of the vibration sense organs have been studied in different species. Most of these studies were made by recording summed potentials from the leg nerve

by means of hook electrodes (AUTRUM, 1941; SCHNORBUS, 1971; DAMBACH, 1972). With this technique, one can only describe the characteristics of whole organs, not of single cells.

Detailed single cell recording of vibration receptor cells in the legs of adult locusts and bushcrickets were performed by KÜHNE (1982). He introduced a physiological classification of the different vibratory receptors; type I represents the different campaniform sensillae, type II probably the joint receptors, type III and IV correspond to the different subgenual receptor cells.

The morphology of the central projections of campaniform sensillae located on the proximal parts of all six legs in adults of *Locusta migratoria* were first investigated by backfilling with CoCl_2 via the dendritic region of these receptor cells (HUSTERT et al., 1981). They showed that the different groups of campaniform sensillae form similar fiber-end branches in the thoracic ganglia of the ventral nerve cord. Similar research on *Gryllus campestris* and *Gryllus bimaculatus* has been done by EIBL & HUBER (1979).

Investigations on the development of the auditory system in larvae of the locust *Locusta migratoria* show a stepwise differentiation of the tympanum and of the cuticular structures it bears (MICHEL & PETERSEN, 1982). All the receptor cells of the tympanal organ are already present in the first instar larvae, but the tympanum is not yet developed. The functional properties of the high-frequency receptor cells in larvae are different from those in adults. They are far less sensitive in the frequency range above 10 kHz than they are in adults. The convergence of auditory and vibratory inputs onto the different ventral cord neurons ascending to the brain could already be found in last instar larvae as in adults (PETERSEN et al., 1982). Therefore morphological and physiological investigations of the different vibratory receptor cells on all legs in the last instar larvae and in adults are of great interest as a basis for further studies in this field.

This investigation compares the location of the different mechanoreceptors in the legs, the physiological properties and the central projection patterns of the receptors of all legs of 5th instar larvae and adults of *Locusta migratoria* and of *Schistocerca gregaria*. The relationship between function and projection of the vibratory receptor cells is considered.

MATERIALS AND METHODS

382 imagines and 148 5th instar larvae of *Locusta migratoria* and 209 imagines of *Schistocerca gregaria* were used for electrophysiological and histological studies.

HISTOLOGY

KOH-treatment of the legs. Legs of imagines and 5th instar larvae were treated with a 10% KOH solution to make the cuticle transparent. The position of campaniform sensillae fields and the number and orientation of individual sensillae were determined using a phase contrast microscope (Wild M20, with drawing attachment).

Cobalt-staining. The location and innervation of receptor cells within the legs could be demonstrated by centrifugal filling of leg nerve with 0.5 M CoCl_2 solution through their sectioned axons. After 24-28 h the leg was soaked in $(\text{NH}_4)_2\text{S}$ (1%) to precipitate CoS and then fixed in formaldehyde (4%). The dehydrated whole-mount preparation was made transparent in styrol to localize the stained neuronal structures within the leg. The central projections of entire nerve branches were investigated by Cobalt backfilling through their sectioned axons. In addition to the treatment described above the preparations of the thoracic ganglia were silver-intensified (after BACON & ALTMAN, 1977). With the help of camera-lucida-drawings the stained central projections could be analysed.

Central projections of individual campaniform sensillae of the different sensillae fields on the legs were revealed by penetrating the caps of single sensil-

lae with glass microelectrodes filled with 3 M CoCl_2 solution. The preparations were treated as described above.

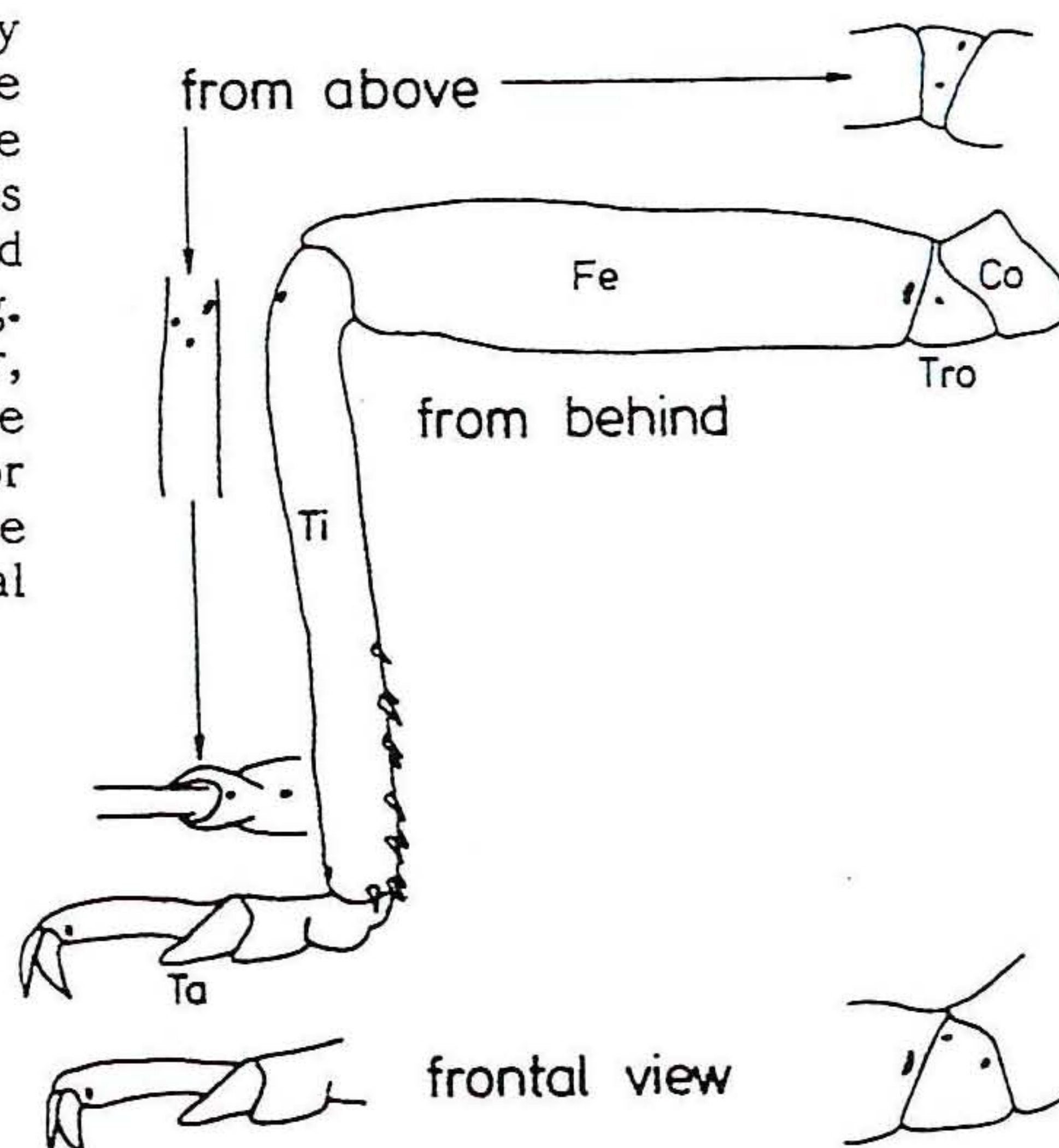
COMBINED EXTRACELLULAR RECORDING AND STAINING. The animals were anesthetized with CO_2 and, after removal of the wings and legs not to be tested, fixed to a metal holder, dorsal side down, with a wax colophonium mixture. The equipment and experimental procedure for the electrophysiological experiments have been described elsewhere (KÜHNE, 1982). The method used by PITMAN et al. (1973) for combined recording and staining, later modified for extracellular recording by REHBEIN et al. (1974), was used to record the responses of, and show the morphology of single neurons from the entrance of the leg nerve to their respective ganglia. The microelectrodes were filled with 3 M CoCl_2 solution (resistance 10-30 M Ω) and the reference electrode was a silver wire inserted in the abdomen. The vibration stimuli were sinusoidal oscillations in the range from 30 to 5000 Hz. Stimulus duration was 100 ms and the repetition rate 2/sec. Intensities were varied between 0.05 m/s^2 to about 5 m/s^2 accelerations. After recording the ganglion was treated with histological methods already described.

RESULTS

TOPOGRAPHY OF VIBRATION RECEPTORS

Campaniform sensillae. On the fore, mid and hind legs the campaniform sensillae are preferentially arranged in groups forming sensory fields. These groups are located in well defined depressions of the cuticle. In addition, there are single campaniform sensillae on the distal parts of the legs. The number of fields and the absolute number of single campaniform sensillae are the same on fore and mid legs but are considerably less on the hind legs (Fig. 1; Table 1). The single ones are predominantly distributed on the tarsi. Another group of single campaniform sensillae can be found in connection with the ventrally situated spines of the tibiae. There they are located at the moveable bases of the spines.

Fig. 1. Distribution of the sensory fields of campaniform sensillae on the mid legs of *Locusta migratoria*. The same arrangement of sensory fields is found on the fore legs. On the hind legs some of the fields are missing. Co, Tro, Fe, Ti, Ta: coxa, trochanter, femur, tibia, tarsus respectively. The whole leg is drawn from a posterior view; some leg parts of interest are also shown from above and in frontal view.



	5th instar				imago			
	fl	ml	hl	Σ	fl	ml	hl	Σ
legs								
number of sensory fields	12	12	6	30	12	12	6	30
total number of campaniform sensillae	116	115	56	287	129	130	64	323
a) in sensory fields	91	88	45	224	104	103	53	260
b) single ones	7	7	7	21	7	7	7	21
c) single ones in connection with tactile spines	18	20	4	42	18	20	4	42

Table 1. Number of sensory fields and of campaniform sensillae on the fore leg (fl), mid leg (ml), and hind leg (hl) of 5th instar larvae and adults of *Locusta migratoria* (indicated only for one side).

The majority of the sensillae on the tibiae are orientated parallel to its longitudinal axis, while this could not be found for sensillae located on the tarsi. HUSTERT et al. (1981) described the arrangement and orientation of campaniform sensillae for the trochanter and femur; our findings confirm their results.

The number and location of the sensory fields and the orientation of campaniform sensillae on the six legs of 5th instar larvae correspond to those in adults. However, except in the tarsi, the absolute number of campaniform sensillae in all fields is less (Table 1).

Subgenual receptors. One subgenual organ lies fan-shaped across the blood channel of the proximal tibia of all six legs. At one side about 15 receptor cells are located at the anterior-dorsal cuticle of the tibia and the organ extends across to its attachment point on the posterior cuticle.

Slightly more dorsally is found a group of about 8 densely packed subgenual receptors ("Nebenorgan" after SCHNORBUS, 1971) similarly orientated to those of the subgenual organ.

In a dorso-ventral view of the tibia another separate group of 8-10 receptor cells can be demonstrated which are located a little more distally. This third part of the subgenual complex of receptor organs is called the distal organ. It is attached distally to a transverse membrane in the blood channel and seems not to be involved in the perception of vibratory signals (SCHNORBUS, 1971).

Fig. 2 shows that all the subgenual receptor-cell axons run via the subgenual nerve (N5B₁ after CAMPBELL, 1961) to the respective ganglion.

Chordotonal organs. The femoral chordotonal organ at the dorsal cuticle proximal in the femur, near the trochanter-femur joint of the fore and mid legs is bipartite (Fig. 2A, B). The axons of about 200 receptor cells in the proximal and 50 in the distal part of the organ (BURNS, 1974) reach the ventral nerve cord via the subgenual nerve (N5B₁). In contrast, in the femur of the hind leg about 35 receptor cells build up the femoral chordotonal organ, which is located in this case 3 mm proximal to the femur-tibia joint. Chordotonal organs at the tibio-tarsal joint (YOUNG, 1970) and within the tarsi (KENDALL, 1970) may as well act as vibration receptors.

Hair sensillae. Even though the hair sensillae seem not to react to substrate vibration they may play an important role in the perception of touch and contact vibration. Their distribution in the proximal parts of the mid leg for instance,

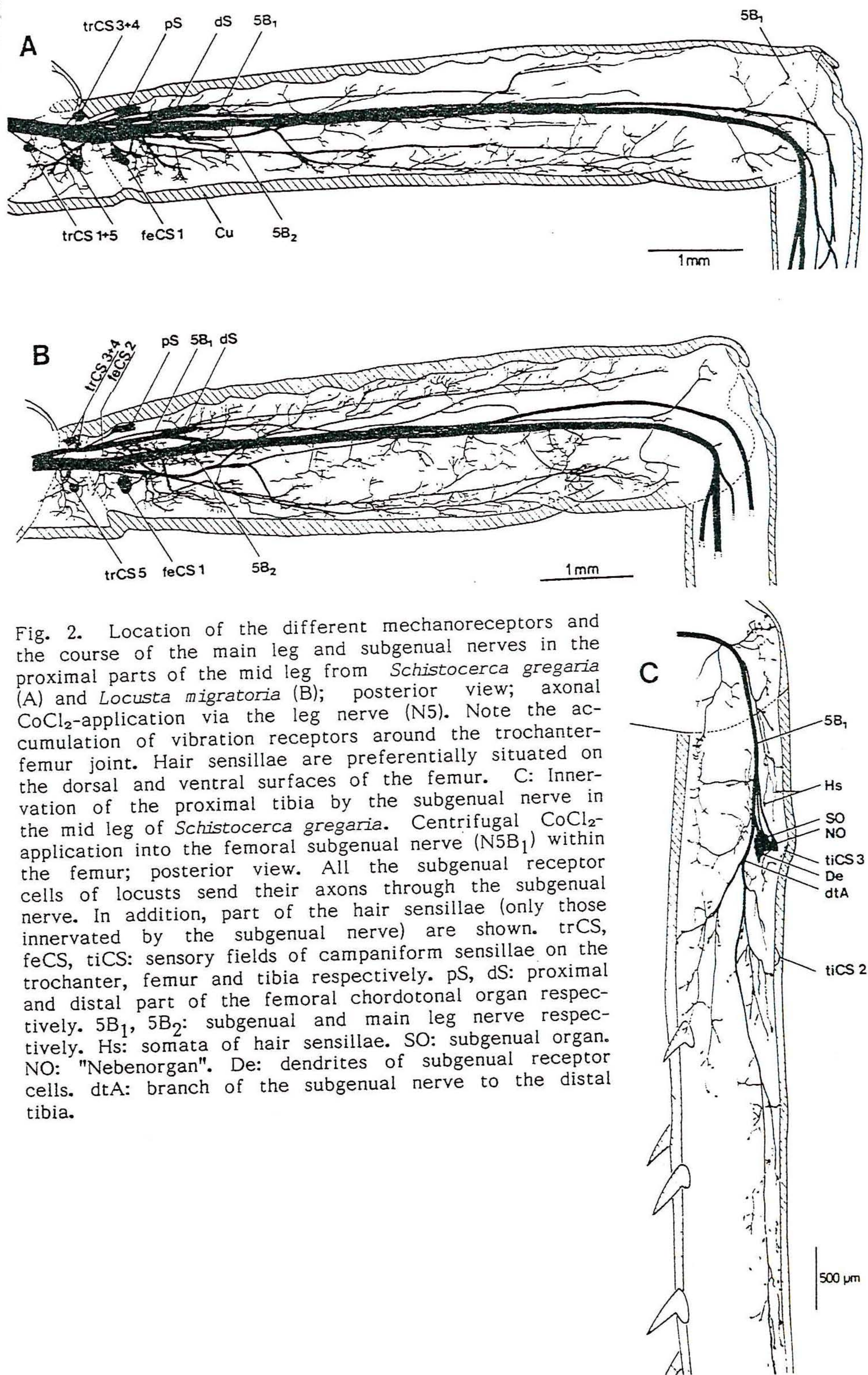


Fig. 2. Location of the different mechanoreceptors and the course of the main leg and subgenual nerves in the proximal parts of the mid leg from *Schistocerca gregaria* (A) and *Locusta migratoria* (B); posterior view; axonal CoCl_2 -application via the leg nerve (N5). Note the accumulation of vibration receptors around the trochanter-femur joint. Hair sensillae are preferentially situated on the dorsal and ventral surfaces of the femur. C: Innerivation of the proximal tibia by the subgenual nerve in the mid leg of *Schistocerca gregaria*. Centrifugal CoCl_2 -application into the femoral subgenual nerve (N5B₁) within the femur; posterior view. All the subgenual receptor cells of locusts send their axons through the subgenual nerve. In addition, part of the hair sensillae (only those innervated by the subgenual nerve) are shown. trCS, feCS, tiCS: sensory fields of campaniform sensillae on the trochanter, femur and tibia respectively. pS, dS: proximal and distal part of the femoral chordotonal organ respectively. 5B₁, 5B₂: subgenual and main leg nerve respectively. Hs: somata of hair sensillae. SO: subgenual organ. NO: "Nebenorgan". De: dendrites of subgenual receptor cells. dtA: branch of the subgenual nerve to the distal tibia.

is shown in Fig. 2A, B. While with all the other mechanoreceptors described above no distinction can be made between *Locusta migratoria* and *Schistocerca gregaria* neither in their number and location nor in their innervation pattern, remarkable differences in the number and distribution of hair sensillae can be found between the two species (Fig. 2A, B). Corresponding to the hairy appearance of *Locusta migratoria* 3-4 times more receptor cells of hair sensillae could be demonstrated in the respective areas of the cuticle. The diameters of the somata of the receptor cells are distinctly smaller in *Locusta migratoria* than in *Schistocerca gregaria*.

PHYSIOLOGY OF THE VIBRATION RECEPTORS. Based on their response characteristics and their threshold curves KÜHNE (1982) subdivided the vibratory receptor cells of the adults into type I (campaniform sensillae), type II (chordotonal

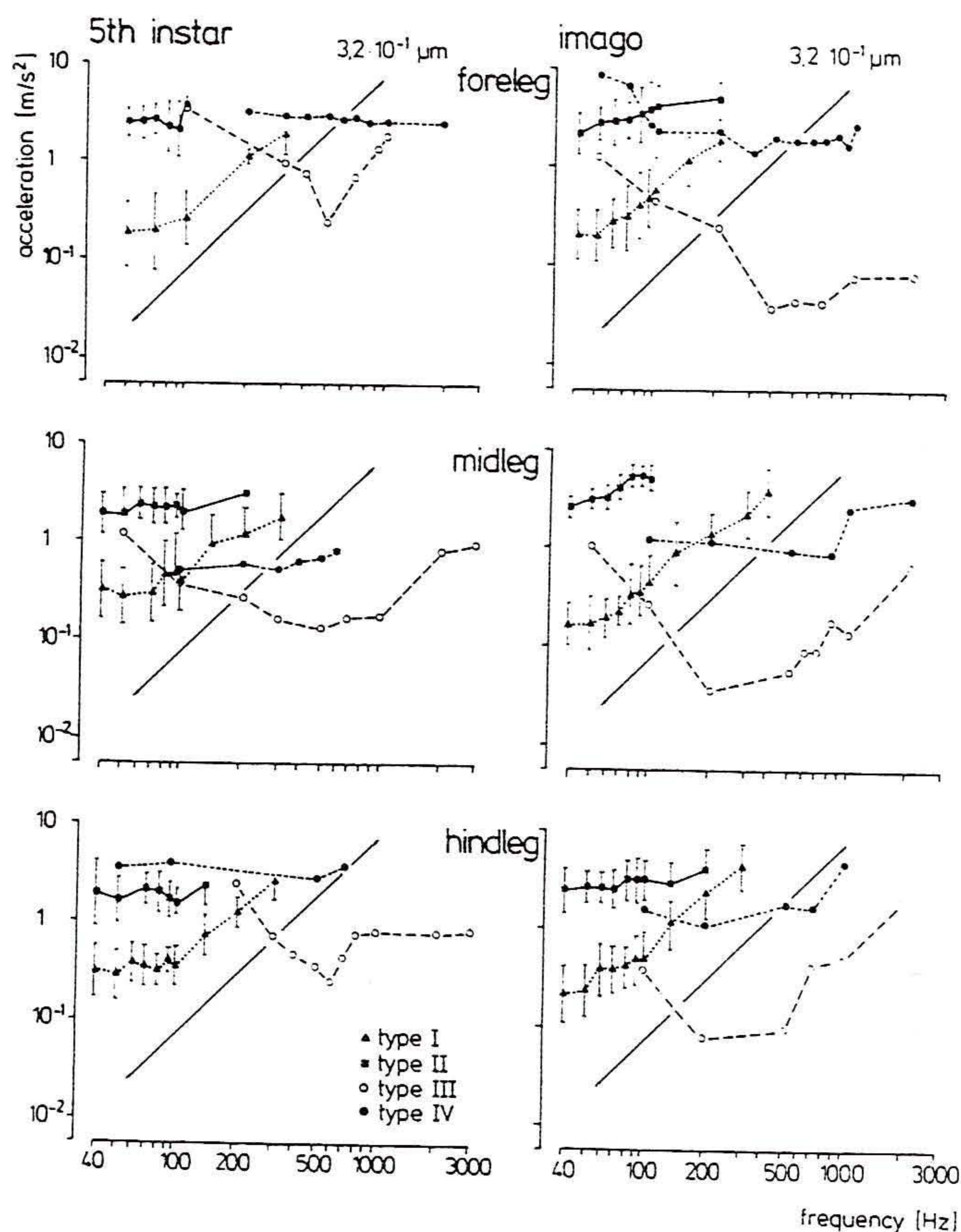


Fig. 3. Threshold curves of the four types of vibration receptors of the fore, mid and hind legs. Comparison of the different vibration receptors between 5th instar larvae and adults of *Locusta migratoria*. All measured points at the different threshold curves represent averaged values of the reactions to at least four identical stimuli. Stimulus duration: 100 ms; repetition rate: 2/s.

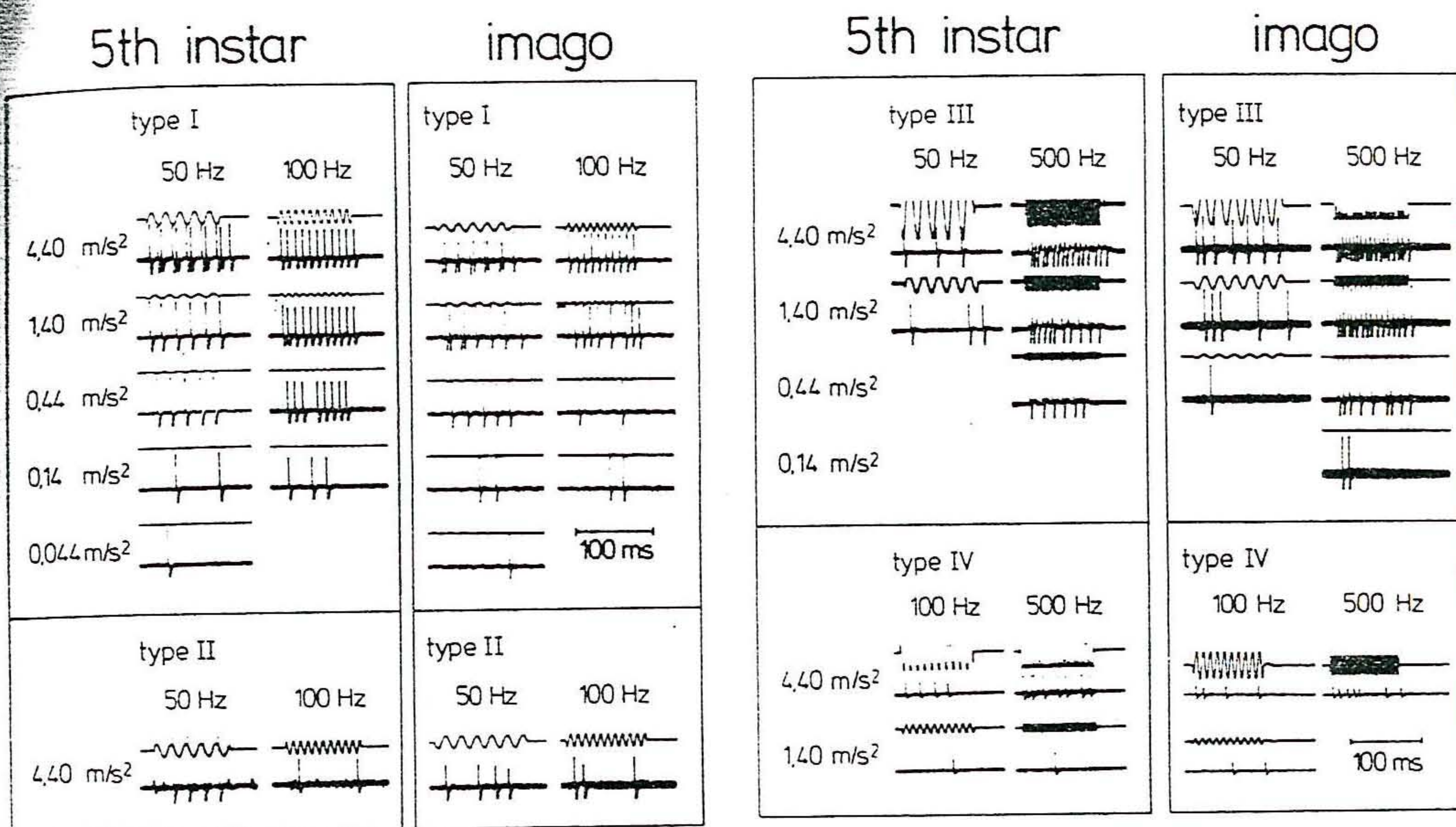


Fig. 4. Responses of type I, II, III, IV receptors of the mid legs of 5th instar larvae and adults of *Locusta migratoria* to vibration stimuli of different frequencies and intensities (given in acceleration values). The upper trace shows the stimulus vibration; the lower trace the reaction. Stimulus duration: 100 ms; repetition rate: 2/s.

joint receptor cells) and type III and IV (subgenual receptor cells). Fig. 3 shows the threshold curves of these receptor cell types for the fore, mid and hind legs in the 5th instar larvae and adults of *Locusta migratoria*. All 4 types are present in the 5th instar larvae; they have the same response characteristics and react within the same frequency ranges as the receptor cells of adults (Fig. 4). Type I and II receptor cells have the same sensitivity in adults and larvae; type III are less sensitive in larvae about 10 dB.

CENTRAL PROJECTIONS OF THE VIBRATION RECEPTORS. Characteristically the projection areas of all four types of vibration receptor cells are limited to the ipsilateral half of their segmental ganglion (Fig 5). The projection areas of acoustic receptors do not overlap with those of vibration receptors. The same projection fields and patterns within the thoracic ganglion have been found in 5th instar larvae and adults. Cells of the same physiological type may show slightly different branching patterns in any one ganglion and between ganglia. In addition, the projection structures of individual campaniform sensillae on the legs are revealed by peripheral application of CoCl_2 . Typical bifurcation patterns of sensillae on trochanter and femur, as described by HUSTERT et al. (1981) were found, whereas sensillae on the tibia never showed such projection shapes.

Neurons of the physiological types III and IV never show bifurcation patterns. In addition, centripetal CoCl_2 -filling of the subgenual nerve (N5B_1) within the femur distal to the junction with afferents on the femoral chordotonal organ

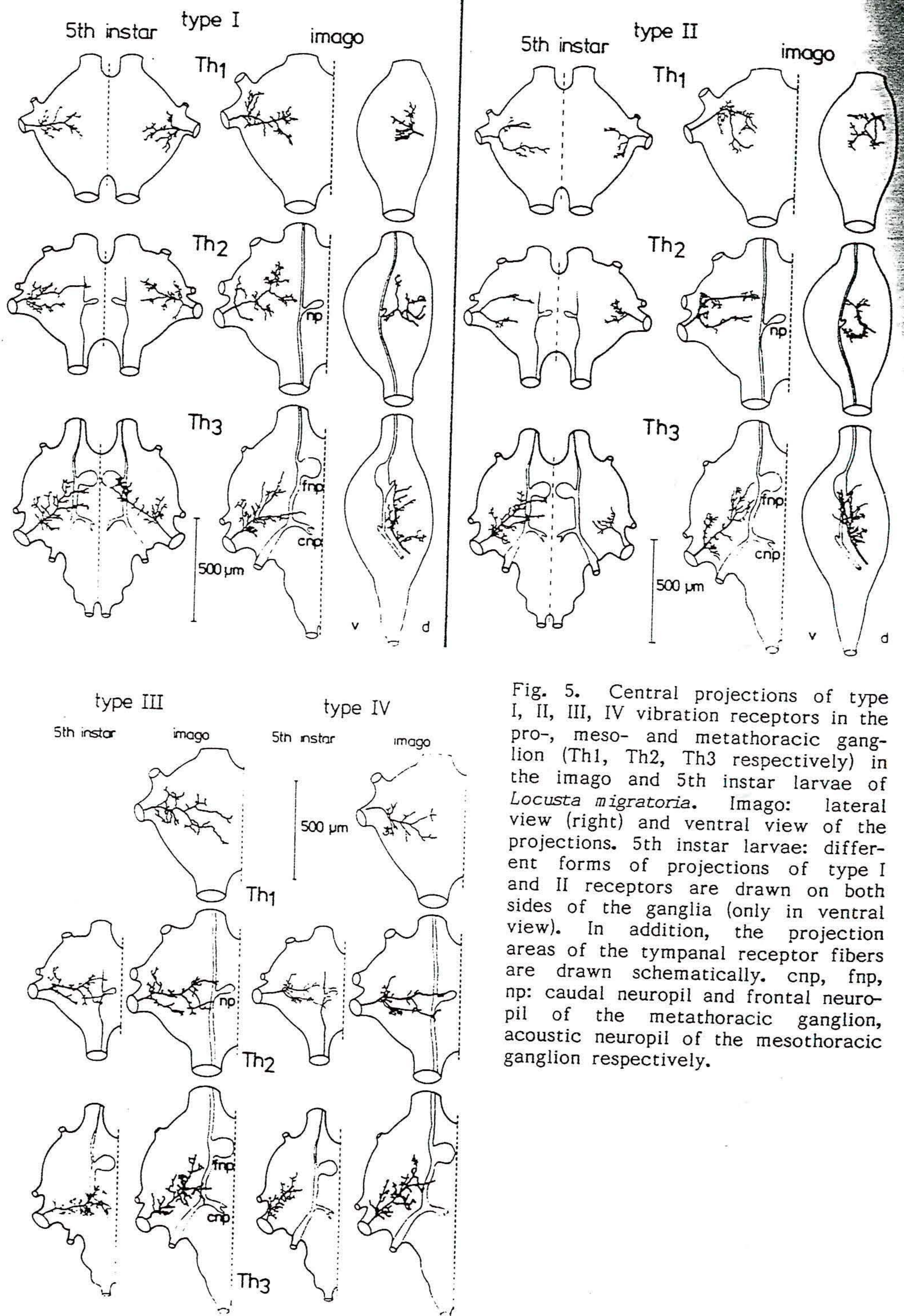


Fig. 5. Central projections of type I, II, III, IV vibration receptors in the pro-, meso- and metathoracic ganglion (Th1, Th2, Th3 respectively) in the imago and 5th instar larvae of *Locusta migratoria*. Imago: lateral view (right) and ventral view of the projections. 5th instar larvae: different forms of projections of type I and II receptors are drawn on both sides of the ganglia (only in ventral view). In addition, the projection areas of the tympanal receptor fibers are drawn schematically. cnp, fnp, np: caudal neuropil and frontal neuropil of the metathoracic ganglion, acoustic neuropil of the mesothoracic ganglion respectively.

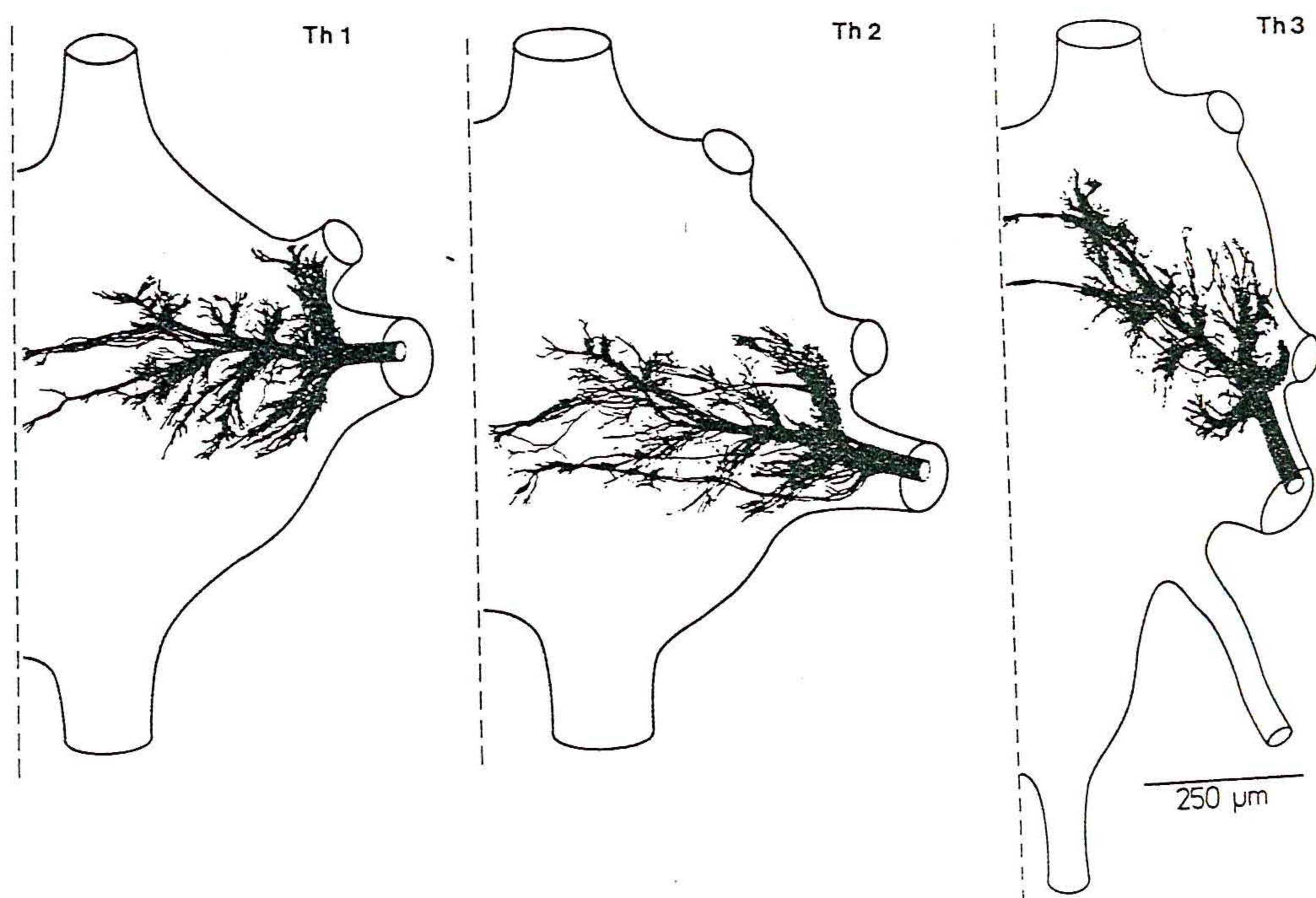


Fig. 6. Central projections of fibers from the subgenual nerve (tibial branch) of the fore, mid and hind leg. Th1, Th2, Th3: pro-, meso- and metathoracic ganglion respectively (adults of *Schistocerca gregaria*).

stains the subgenual receptor axons. Except the subgenual afferents this branch of the subgenual nerve contains axons of hair sensillae (e.g. about 150 in *Schistocerca gregaria*) distributed on the tibia (Fig. 2C). In the proximal part of the tibia they are arranged preferentially on the dorsal and ventral surface, while distally of the most proximal tibial spine only a small nerve branch continues down to the distal tibia. No hair sensillae on the tarsi are innervated via the subgenual nerve. Backfilling (with CoCl_2) of the subgenual nerve within the femur (Fig. 6) stains the subgenual afferents and those of the tibial hair sensillae. The sum of central projections of axons enclosed within the tibial branch of the subgenual nerve therefore includes those of a large number of hair sensillae. Nevertheless mainly neurons with projection patterns like those of type III and IV neurons were stained by backfilling the tibial branch of the subgenual nerve (Fig. 2C). Hair sensillae and subgenual receptors may have similar central projections and therefore differences may not be conspicuous. It is more likely, however, that the stain failed to penetrate the central projections of hair sensillae. With the use of relatively high CoCl_2 -concentration (0.5 M) axons with small diameters, like those of hair sensillae, are difficult to fill (ALTMAN & TYRER, 1980). Therefore the presented central nervous stainings probably show exclusively the sum of central projections of the subgenual receptor cells. The remarkable

coincidence of the central projections of type III and IV receptor neurons with those of subgenual receptors confirms the classification of type III and IV neurons as subgenual receptor cells.

DISCUSSION

This investigation shows that the vibratory receptor system is already well developed in the 5th instar larvae of *Locusta migratoria*. There are no significant differences in the physiology or in the central projection patterns of the receptor fibers in larvae of 5th instar and adults.

However, the number of campaniform sensillae is smaller on the legs of 5th instar larvae than in adults (by about 14%). The majority of campaniform sensillae is located on the trochanter and on the proximal part of the femur. Campaniform sensillae are engaged in reflex movement control (SPINOLA & CHAPMAN, 1975). Their concentration on the proximal leg regions and proximally on the tibia near the insertion points of leg muscles may provide proprioceptive information about cuticular deformation caused by muscle activity during locomotion.

The comparison of central projections revealed from combined recording and staining with those obtained by peripheral iontophoresis of single campaniform sensillae confirms the classification of type I neurons as receptor cells of campaniform sensillae. Whether type II neurons with similar projection patterns are also receptors of campaniform sensillae or of those of the chordotonal organs must await further investigations.

The central projection of subgenual receptor afferents shown by staining the distal branch of subgenual nerve coincide with central projections of type III and IV neurons revealed by combined recording and staining techniques. This may confirm the suggestion of KÜHNE (1982) that type III and IV neurons are subgenual receptors. Whether both physiological types correspond to neurons of different sections of the subgenual complex of receptor organs has to be proved.

COKL et al. (1977) showed that in most cases no direct synaptic connections occur between vibration receptor cells and bimodal vibratory-auditory ventral cord neurons ascending to the brain. Therefore interneurons must be intercalated. Our histological results support this view: 1) Fibers and branches of all receptor types are limited to the ipsilateral half of the respective thoracic ganglion (Fig. 5, 6). 2) To date, no direct contact to the dendritic regions of the ventral cord vibratory-auditory neurons nor to the acoustic neuropil regions of the tympanal receptors have been demonstrated.

From these results one can conclude that the information of the vibration receptor cells (besides that which is engaged in the reflex control) is transmitted via segmental and/or intersegmental interneurons of the above mentioned bimodal ventral cord neurons. In locusts, COKL et al. (1977) could confirm the presence of such interneurons in neurophysiological experiments.

Acknowledgements. We thank Mrs. E. SCHÄFER for technical help. Supported by the Deutsche Forschungsgemeinschaft.