J Comp Physiol A (1991) 168:63–73

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

The giant fiber system in the forelegs (whips) of the whip spider *Heterophrynus elaphus* Pocock (Arachnida: Amblypygi)

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Accepted October 8, 1990

Summary. The front legs of the whip spider *H. elaphus* are strongly modified to serve sensory functions. They contain several afferent nerve fibers which are so large that their action potentials can be recorded externally through the cuticle. In recordings from the tarsus 7 different types of afferent spikes were identified; 6 additional types of afferent spikes were discriminated in recordings from the tibia and femur. Most of the recorded potentials could be attributed to identifiable neurons serving different functions. These neurons include giant interneurons and giant fibers from diverse mechanoreceptors such as slit sense organs, trichobothria, and a joint receptor. In the present report these neurons are characterized using electrophysiological and histological methods. Their functions are discussed in the context of the animal's behavior.

Key words: Giant fibers – Giant neurons – Mechanoreceptors – Whip spider

Introduction

For nocturnal animals, such as whip spiders, chemical and mechanical stimuli provide the major source of information about the surroundings. For whip spiders, the modified front legs (whips) play the most important role in sensing these stimuli. The whips possess a rich array of receptors (Foelix et al. 1975; Igelmund 1984, 1987, 1988), and they are no longer used for walking. Compared to the other legs the whips are greatly elongated. In *Heterophrynus elaphus*, which has a body length of about 3 cm, the front legs reach a length of 26 cm. Electron microscopic studies of the tarsus of a closely related species, *H. longicornis*, revealed several giant nerve fibers with diameters of up to 20 μm in addition to ca. 20000 thin axons. The giant fibers have somata lying peripherally in particular segments of the tarsus; their dendrites are contacted by numerous synapses (Foelix 1975; Foelix and Troyer 1980). To date nothing is known about the physiology of these giant neurons. The analogy with central giant neurons in other arthropods (cockroaches, crickets, crayfish) suggested that they play a part in escape responses.

In the context of this putative function, the goal of the present research was to study the whips of *H. elaphus* in order to describe the physiology and morphology of the giant fibers and to relate their function to the sensory inputs on the one hand and to the animal's behavior on the other.

Only 10 animals were available for the physiological experiments, so it was not feasible to attempt intracellular recordings or backfills with cobalt chloride or fluorescent dyes. Since giant neurons with somata located in the periphery are a unique feature, only found in whip spiders and whip scorpions (Foelix and Troyer 1980), it seemed important to investigate their physiology as far as possible. Therefore we developed methods which allowed multiple recordings to be made repetitively from intact animals.

Material and methods

Experimental animals. The experimental animals were whip spiders of the species *Heterophrynus elaphus* Pocock collected in the jungle near Pucallpa, Peru in 1980, 1981 and 1983. Electrophysiological experiments were performed on 9 adult animals and one nymph. In the laboratory the animals were kept separately in glass boxes (30 × 25 × 50 cm). The top and one side of the boxes were lined with cork, and a cork tube provided a refuge. The temperature ranged from 20 to 24 °C; the humidity was kept at 90 to 100% with the aid of a water-soaked, plastic mat (Moosy; Fa. Compo, Münster, FRG) standing in a water reservoir. Live prey in the form of 1 or 2 crickets was continuously available in the box.

Abbreviations: *GN* giant neuron; *S* segment

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Histology. Two whips from different animals were amputated after the electrophysiological investigations and fixed in 5% glutaraldehyde in cacodylate buffer. For fixation the whips were cut into pieces of 2–5 mm length. In addition the cuticle was sliced open at intervals of less than 2 mm in order to minimize the diffusion distance. The specimens were post-fixed in 1% osmium tetroxide. After dehydration in an acetone series the specimens were embedded in epoxy (medium 1 of Spurr 1969). Sections were prepared from segments of the tarsus shown to be of interest in the electrophysiological tests. Longitudinal and cross-sections 0.7 to 1.5 μm thick were cut with glass knives on a Reichert OmU3 ultramicrotome. The sections were treated with periodic acid and stained with methylene blue/Azur II (Richardson et al. 1960). They were examined with a Leitz Ortholux microscope. Ultrathin sections (80 nm) cut with a diamond knife were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 9A electron microscope. Circular axon diameters were calculated on the base of cross section areas determined from EM micrographs with a Zeiss Morphomat 30. For scanning electron microscopy ultrasonically cleaned and air-dried preparations were sputtered with gold and examined with a Hitachi S-520

Electrophysiology. Live whip spiders are difficult to obtain and *H. elaphus* has not yet been successfully bred in the laboratory. As only 10 animals were available for the physiological experiments, we were limited to using methods that did not injure the animals. With these techniques the animals were investigated repetitively in altogether 79 experimental sessions.

For each experimental session an animal was anesthetized with carbon dioxide for 2 min and restrained with u-shaped insect pins on a dissecting board so that it could not move. In some experiments a part of the investigated whip was left unrestrained. To prevent dehydration the animal was covered with moist Kleenex.

The action potentials were recorded through the cuticle by placing paired electrode wires (100 μm silver wire or simply the bent pins of a 14 pin IC plug) loosely against the whip. The separation of the paired electrodes for the differential recording was 1.25 or 2 mm. Electrical contact was established through a small ring of electrode cream (Hellige), which could easily be washed off after the experiment. The recorded signals were amplified and stored on tape using a Racal Store 7 tape recorder. For analysis the recorded signals were displayed using a Gould Brush 440 pen recorder or processed through a 7 channel interface connected to a PDP 11/40 computer.

Mechanical stimulation of single hairs was performed using minute insect pins fixed to a piezoelectric crystal (PXE multimorph, Valvo) or to a mini-shaker (Brüel & Kjaer 4810). For quantitative movements of single tarsus segments the whip was placed in a 35 mm long, 240 to 300 μm wide and 600 μm deep groove in a plexiglas holder. This holder was divided into two parts separated by 150 μm. Using a Brush Instruments pen motor, the distal part could be deflected by $\pm 30°$ with respect to the proximal part. The tarsus was pushed into the groove with a fine hair and held there by the pressure of the deflected lateral bristle hairs.

Terminology. The anatomical axes are defined here for a situation in which the whips are fully extended laterally and perpendicular to the body axis. With this definition, the blood vessel in the tarsus lies ventrally, as it does in spiders. The segments of the tarsus and the tibia are numbered from distal to proximal. Segment occasionally is abbreviated as S.

Results

Histology

Sensilla. In comparison to the walking legs, the whips of the whip spiders are very long and thin (Fig. 1) and, due to extensive segmentation, very flexible. In *H. elaphus* the tarsus normally contains 74 segments and the tibia 33 segments. The whips carry 8 types of sensory hairs (bristles, club sensilla, two types of porous sensilla, two types of rod sensilla, leaf-like hairs and trichobothria), 4 types of slit sense organs and 3 single sensilla of unknown function (plate organ, pit organ, modified claws). As the sensilla were described in detail previously, here the description will be limited to that necessary for an understanding of the giant fiber system. For further information see Igelmund (1984, 1987, 1988).

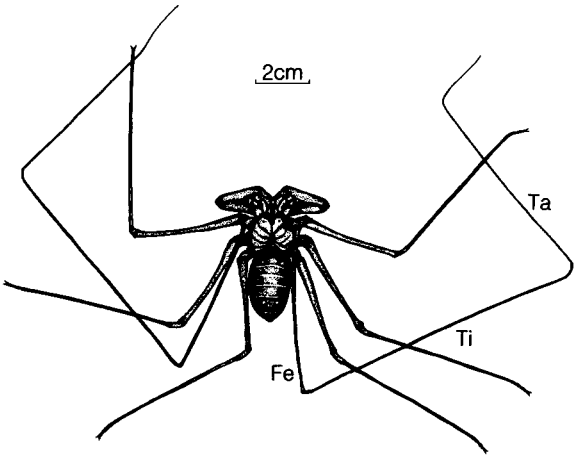

Fig. 1. Dorsal view of *Heterophrynus elaphus*. In resting position, the femur (*Fe*) of the whip is held backwards, the tibia (*Ti*) sidewards, and the tarsus (*Ta*) forewards or deflected parallel to the tibia. Tarsus and tibia are very flexible because of their segmentation but active movements at the segment boundaries are only possible in the distal part of the tarsus. In the specimen shown, the left whip has been regenerated after autotomy and is therefore smaller than the right one

Bristles (Fig. 2a, b) are present on all segments of the tarsus and tibia as well as on the femur. With lengths of up to 1 mm they are the longest hair sensilla on the whip. In adult animals approximately 1700 bristles are present on the tarsus alone. Each bristle contains 8–12 dendrites which continue through the hair shaft to a terminal pore, and 2 dendrites which form large tubular bodies and terminate at the base of the hair. This innervation pattern suggests a combined chemo- and mechanoreceptive function.

Trichobothria are found only on the tibia of the whip. A total of seven hairs are distributed in a fixed pattern on tibial segments 1, 2, 3, 4, and 13. The trichobothria are mechanoreceptors. They are flexibly set in a cup-shaped socket and are deflected by very slight air currents. In spiders and scorpions trichobothria are innervated by a variable number of receptor cells (up to 7–8; Reißland and Görner 1985). In the present study the internal structure and the innervation pattern of trichobothria have not been investigated.

Slit sense organs on the tarsus of the whip occur only as single slits. These can be divided into 3 types according to size and form. Type I (Fig. 2b) is represented by a single organ on the caudal surface of S 22. With a length of 70–80 μm it is the largest slit organ. Three slit organs of type II are located on S 1, 19 and 20 of the tarsus; these

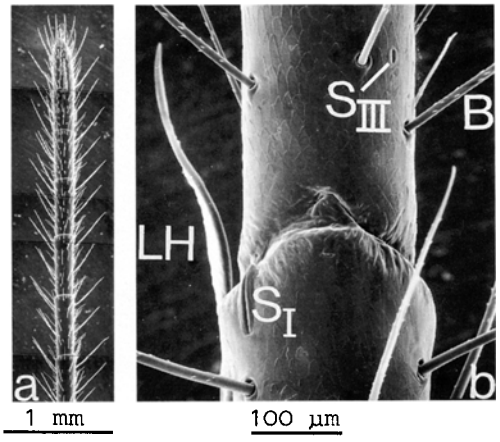

Fig. 2a, b. Morphology of the whip tarsus. **a)** Overview of the distal end of the whip showing segments 1 to 6. **b)** Dorsal view of a left whip showing the segment border between S 21 and S 22, numerous bristles (*B*), a leaf-like hair (*LH*) seen from the thin side, a slit sense organ of Type III (*S III*) as well as the large slit sense organ of Type I (*S I*) on the segment border. This segment border, in contrast to all others on the whip tarsus, is not circular but runs at an angle to the whip axis and the proximal segment possesses a projection inserting into the distal segment

slits are ca. 20 µm long (Fig. 3d). The third type (Fig. 2b) is represented by 1 to 3 slits on each tarsus segment. For this type the slit length ranges from 9 to 13 µm. Type III slit organs are also present on the tibia. Each slit is innervated by 2 sensory neurons (studied here only in Types I and II).

Two large sensory neurons, in addition to the two neurons innervating the large slit organ, are present in S 22: we assume that they are *joint receptor* cells. Each possesses a distal dendrite which leads to the joint membrane on the caudal side of the tarsus (Fig. 3e) and branches there in the hypodermis as is typical for joint receptors of spiders (Foelix and Choms 1979; Seyfarth 1985).

Internal structure of the whip

The outer diameter of the 10 cm long whip tarsus is ca. 320 µm in proximal segments and narrows to 180 µm in distal segments. Cross sections (Fig. 3a, b) show the hypodermal tissue (Hy), which contains the receptor cells and glands, the ventral blood vessel (BV), a dorsal and a ventral tendon (Te), the two tarsus nerves (N1, N2) and a large lumen (Lu) through which the haemolymph returns from the leg tip to the body. As shown in Fig. 3a, b, the anterior and posterior nerves are designated N1 and N2, respectively. This corresponds to the terminology for *H. longicornis* implicit in Fig. 1b–3 of Foelix and Troyer (1980) (Foelix, pers. comm.).

In S 21, 14 mm from the tarsus tip, N1 and N2 contain each approximately 8000 fibers, as counted from EM micrographs of one preparation. An estimation based on the distribution and average innervation pattern of the different receptor types (Igelmund 1987) reveals that about 3000 of these axons belong to bristle hairs, about 10000 to porous hairs, and 2000–3000 to the other types of sensilla present between the tarsus tip and S 21 (club sensilla, rod sensilla, slit sense organs, pit organ, plate organ). In S 72, 4.5 mm distal to the tibia-tarsus-joint and 90 mm from the tarsus tip, cross sections of the same preparation contain ca. 16500 and 13000 fibers in N1 and N2, respectively. The ca. 8500 (N1) and 5000 (N2) fibers added between S 21 and S 72 belong almost exclusively to bristle afferents except ca. 500 axons belonging to slit sense organs and leaf-like hairs. As the bristles located on S 72–S 74 are innervated by approximately 500 sensory cells, the whip tarsus contains about 30000 fibers at the tibia-tarsus joint. The diameter of most axons is in the range of 0.1–0.2 µm.

In sections from proximal segments of the tarsus (Fig. 3b), three large-diameter axons are present in N1. The two largest axons of the tarsus, marked with asterisks in Fig. 3b, will be referred to as GN1 and GN2. They attain diameters of 21 µm and 14 µm, respectively; the third axon reaches a diameter of 11 µm. In the posterior nerve, N2, the five largest axons attain diameters of 7–8.5 µm in the proximal tarsus. These axons are smaller than GN1–GN3 but nevertheless distinct from the other fibers in N2 (Fig. 3b). Therefore, these 5 fibers are also classed as giant fibers. The diameters of the 50 largest axons of each nerve in S 72 are shown in Fig. 4. In cross sections from distal tarsus segments (but proximal to S 5), only one giant fiber, namely GN1, is prominent in the anterior nerve (Fig. 3a). Its diameter is ca. 8 µm in S 21. The number of giant fibers as well as their diameters increase from distal to proximal. The somata belonging to the giant fibers are located in particular tarsal segments. With a length of ca. 150 µm and a diameter of ca. 50 µm the soma of GN1 is the largest neuronal cell in the tarsus (Fig. 3c). This cell is located in S 5. The somewhat smaller soma of GN2 is in S 23. GN1 and GN2 are two interneurons which receive inputs from all the bristles of the tarsus; their characteristics will be described in a separate report (Igelmund and Wendler 1991).

Additional large somata are present in S 19, 20 and 22 of the tarsus. These somata are considerably smaller than those of GN1 and GN2; they have lengths of 40–80 µm and diameters of 20–35 µm. As can be seen in longitudinal sections (Fig. 3d, e), these cells are the pairs of receptor cells belonging to the slit organs in the respective segments and the two neurons in S 22 considered to be joint receptor cells (see above). Like GN1 and GN2 these cells show a homogeneous nucleus and a clearly defined nucleolus; in contrast the nuclei of the numerous small sensory cells are rich in chromatin. The large somata lie caudally in the tarsus adjacent to nerve N2 and the axons presumably run in this nerve. In contrast to GN1, which is always discriminable as the largest axon in N1, the axons of these neurons cannot be followed in the nerve without specific staining or serial sections. However, the electrophysiological results described below suggest that the axons from 2 of the 4 large somata in S 22 belong to the group of large fibers in nerve N2.

Somata forming a third size class are present in many segments. These cells have lengths of 30–40 µm and diameters of ca. 15 µm. They are still considerably larger

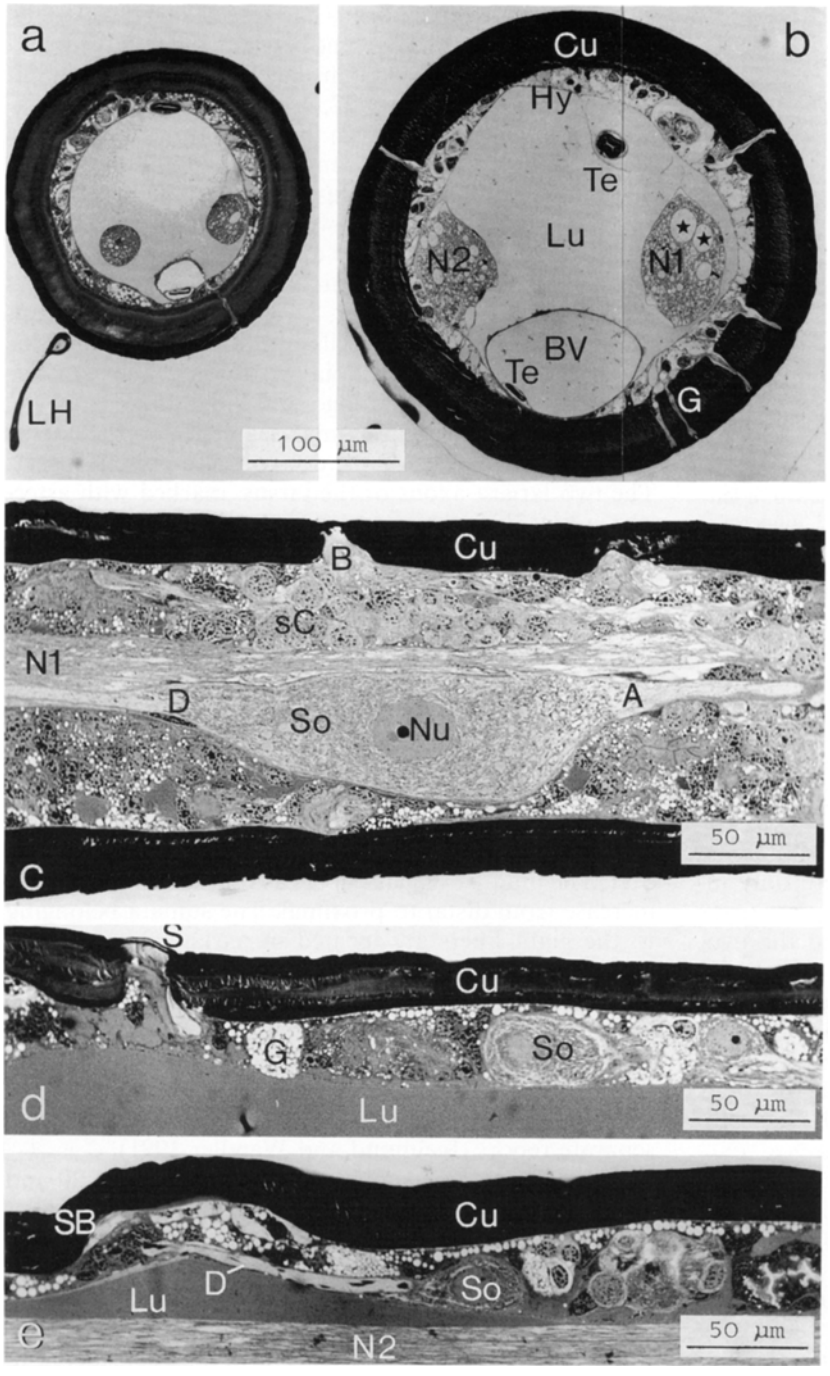

Fig. 3a–e. Sections through the whip tarsus. Cross sections through S 21 (**a**) and S 72 (**b**) together with longitudinal sections through S 5 (**c**), S 20 (**d**) and S 22 (**e**). Posterior is to the left in **a** and **b**; distal is to the left in **c–e**. The longitudinal sections show the soma of interneuron GN1 (**c**), a slit sense organ of Type II with one of the two primary sensory neurons (**d**, the dendrite running to the slit is out of the plane of section), and a putative joint receptor cell (**e**). *Abbreviations: A* axon, *B* base of a bristle, *BV* blood vessel, *Cu* cuticle, *D* dendrite, *G* gland opening (in **b**) or gland cell (in **d**); *Hy* hypodermis, *LH* leaf-like hair (in cross-section); *Lu* lumen, *N1* anterior nerve, *N2* posterior nerve, *Nu* nucleus, *S* slit sense organ, *SB* segment boundary, *sC* sensory cells, *So* giant soma, *Te* tendon. The stars in **b** mark the two largest giant fibers (GN1 and GN2) in nerve N1

than those of the many small sensory cells and also differ from the latter in possessing a homogeneous nucleus. These somata presumably represent the receptor cells of the Type III slit organs (Igelmund 1987).

Electrophysiology

With external electrodes placed loosely against the cuticle, 7 types of afferent spikes were discriminated in recordings from the whip tarsus; 5 of these could be elicited by selective stimuli. In recordings from the tibia and femur 6 additional types of afferent spikes were identified. These also could be selectively elicited. The amplitude of the largest action potentials exceeded 2 mV, far above the noise level.

To demonstrate the discrimination parameters and all the types of tarsal spikes together, Fig. 5 shows an example of simultaneous recordings from 4 positions on the whip tarsus during mechanical and chemical stimulations. This recording illustrates 3 features. First, a single recording from the proximal part of the whip is sufficient to discriminate the two largest types of action potentials on the basis of their amplitude. However, several simultaneous recordings are necessary for the identification of the smaller action potentials because the recorded am-

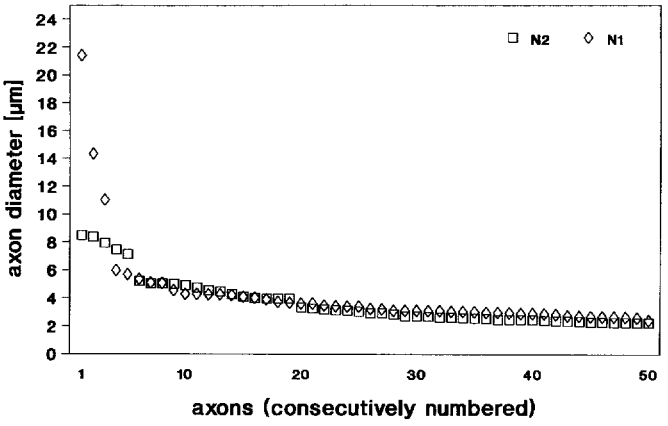

Fig. 4. Axon diameters in N1 and N2. The diagram shows the diameters of the 50 largest axons in N1 and N2, respectively, as calculated from the axon areas in a cross section from S 72, 4.5 mm distal to the tibia-tarsus joint

plitude varies from place to place. Second, not all action potentials can be recorded from all segments. Action potentials of Types 2, 6 and 7 first appear in the second recording trace. Third, the action potentials of different types arise in different segments. The action potentials of Types 1 and 5 propagate proximally at all recording sites, while at the most distal recording site those of Types 3 and 4 propagate distally as judged on the basis of polarity and – here apparent only for Type 3 – on the conduction time between the two distal recording sites. This means that these action potentials originate between the first and second electrode pairs.

Analysis of several such records of each animal led to the following 3 criteria for differentiating action potentials: 1) the amplitude and the conduction velocity in different regions of the whip, 2) the place of origin and the presence of action potentials in different segments, and 3) the adequate stimulus.

Amplitude and conduction velocity alone are not sufficient for an across-animal comparison of the units other than Type 1 and Type 2, because these parameters vary both in different regions of the tarsus and from one animal to another, but the use of the other criteria listed above allows a clear identification of even the smaller action potentials. The constancy of amplitudes and conduction speeds at constant recording sites as well as the lack of superposition of spikes of the same type suggest that the different types of action potentials can each be attributed to a single giant fiber.

In the following sections the giant fibers producing the different action potentials are described in more detail. The giant fibers of the tarsus are numbered GN1 to GN7 in accord with the numbers assigned to the different electrophysiological units in Fig. 5.

GN1 and GN2: giant interneurons

The action potentials of Types 1 and 2 are the fastest propagating and largest potentials which were recorded from the tarsus. The conduction velocities in the tarsus reach 6 m/s. Their characteristics allow a clear assignment to the two largest giant fibers in the tarsus, interneurons GN1 and GN2. Spikes of Types 1 and/or 2 are elicited by mechanical stimulation of any bristle of the tarsus: deflection of a single bristle is sufficient. The morphology and physiology of these two interneurons are described in a separate report (Igelmund and Wendler 1991).

GN6 and GN7: giant sensory cells

In the freely moving tarsus passive deflection distal to S 22 elicits action potentials of Types 6 and 7; deflection proximal to S 22 is ineffective. The adequate stimulus is bending the tarsus between S 21 and S 22 (Fig. 6).

Sinusoidal movement of the S 21/22 joint leads to alternating bursts of action potentials in GN6 and GN7. For displacement in the rostrocaudal direction the activity in GN6 occurs during deflection caudal from the middle position while the activity in GN7 occurs during rostal deflection (Fig. 6b). In each case the activity begins approximately at the middle position (phase values of 90°

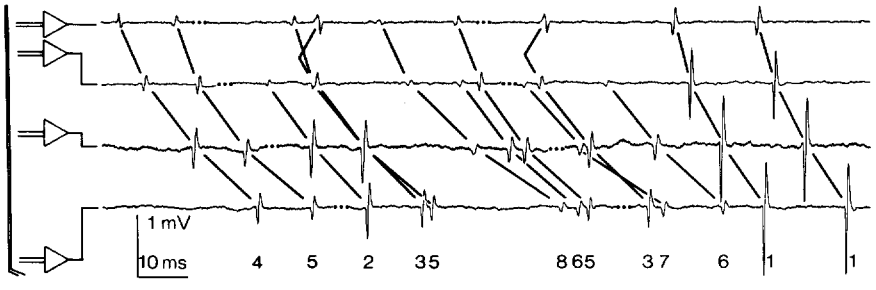

Fig. 5. The types of potentials in the whip tarsus. Drawing on the left shows the tarsus with the tibia-tarsus joint at the bottom and indicates the position of the 4 pairs of bipolar electrodes. Traces to the right illustrate simultaneous recordings from the tarsus during unspecific stimulation (touching with a brush and exposure to air puffs and to odors). This figure contains 3 sections taken from a longer continuous recording to show all 7 types of tarsal potentials described in this paper. Potentials appearing in several traces are connected by lines to indicate conduction direction and velocity. The various potentials are numbered according to their amplitudes in the proximal recording (bottom trace). Numbers 1–7 correspond to the designations of the spike types in the text. In addition to these potentials, smaller spikes of various units appeared (one is shown here as number 8) which were not individually identifiable. See text for further explanation

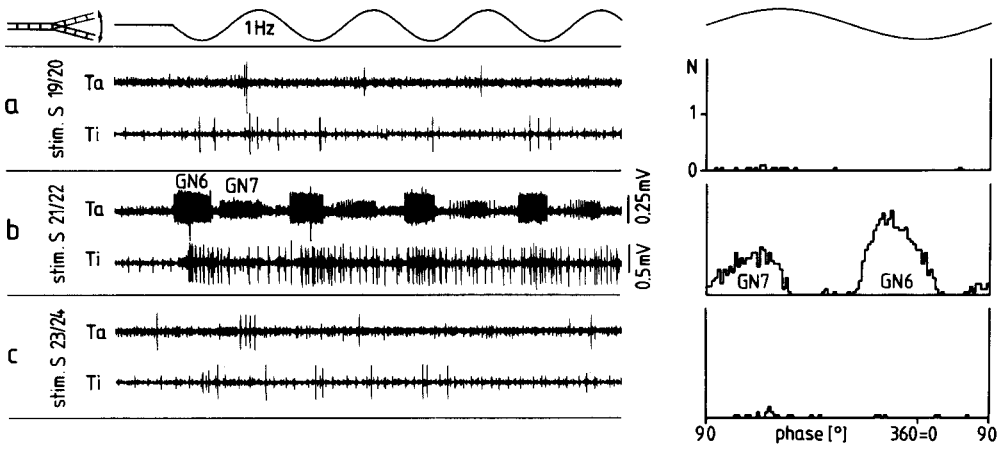

Fig. 6a–c. Activity of GN6 and GN7 in response to selective movement of individual segment borders. The drawing at the top shows the stimulus, upwards corresponding to anterior movement of the distal segments. The movement was applied horizontally with an amplitude of ±5° and a frequency of 1 Hz. Recordings were made from tarsus segment 49 (*Ta*) and from tibia segment 21 (*Ti*) during sinusoidal movement applied to the border between S 19 and S 20 (**a**), S 21 and S 22 (**b**) and S 23 and S 24 (**c**). The histograms at the right show the distribution of potentials of GN6 and GN7 in the recording from the tarsus as averaged over 20 stimulus periods in each stimulus situation. Abscissa: one stimulus period (360°) divided into bins of 3.6°. The posterior extreme point is defined as 0°. Ordinate shows the number of counts/bin/period (N) for spikes of GN6 and GN7. – Bending at the border between segments 21 and 22 elicits a stimulus-specific response in GN6 and GN7 (b); the same stimulus applied at other positions causes only a few scattered potentials probably due to a slight mechanical transmission to the neighboring segments (**a, c**). The spikes in the tibia records (lower traces in **a-c**) represent activity of motoneurons. This activity is increased as a result of the GN6 activity (**b**) (see chapter: Motoneurons)

and 270°) and ends shortly after the maximum deflection. (The posterior extreme point is defined as 0°.) The activity is altered little by changing the plane of movement through a large range of angles (Fig. 7). The phase of the response only changes when the inclination exceeds about 40° in the anterior direction. For an inclination of 30°, GN6 is activated during the dorsocaudal movement; for an inclination of 60° it responds during the rostroventral movement. GN7 shows the reverse pattern. The movement plane at which the phase reverses (ca. 40°) coincides with the line from the center of the tarsus through the slit sense organ of Type I at the segment border.

The action potentials of Types 6 and 7 always arise in S 22. They can be recorded from the tarsus everywhere proximal to S 22. In recordings distal to S 22 these action potentials are not present (Fig. 5). Therefore we assume that the somata of the neurons generating these potentials lie in S 22. Four large somata, belonging to the slit sense organ Type I and to a putative joint receptor, respectively, are present in this segment (see above; Fig. 3d, e); hence these findings suggest that the potentials of Types 6 and 7 are generated either by the two receptor cells of the slit sense organ or by the two putative joint receptor cells or one type of potential by each type of receptor.

Whichever organ is responsible, it is so sensitive that the mechanical stimulation accompanying the rapid deflection of a single bristle in S 1 often suffices to elicit several action potentials in GN6 or GN7. For sinusoidal bending of the joint between S 21 and S 22 at a frequency of 0.5 Hz, the threshold lies at a stimulus amplitude of ca. 0.005° and the response saturates at a stimulus amplitude of about 10°. Small step stimuli of 0.5° elicit phasic activity lasting no more than 200 to 500 ms. For larger steps of 5°, the responses may last for 30 s.

The conduction time for GN6 and GN7 spikes from S 22 to the CNS, as extrapolated from measurements in the femur, is about 65–70 ms and 70–75 ms, respectively. The conduction velocity begins with about 2 m/s and increases proximally; at the proximal end of the tarsus it reaches values of 3.4 m/s (GN6) and 3.1 m/s (GN7). The latter values are equal to the average velocities over the whole length of the whip. In different individuals the mean conduction velocities ranged from 3.3 to 3.7 m/s for GN6 and 2.8 to 3.4 m/s for GN7.

GN3, GN4 and GN5: giant neurons of unknown function

Action potentials of Types 3 and 4 appeared only spontaneously in the records. A reliable stimulation of these units could not be achieved by mechanical, chemical or thermal stimuli, so the adequate stimulus is unknown. As a result it was impossible to systematically investigate these neurons.

Neuron GN5 is selectively activated by blowing tobacco smoke onto the distal segments of the tarsus. A brief exposure (ca. 1 s) to smoke elicits a high-frequency discharge beginning 5 to 15 s after the stimulus onset and reaching ca. 30 spikes/s, which then slowly decays over several minutes. A second burst of activity can only be elicited after several hours. Without smoke stimulation, single action potentials of type 5 occurred spontaneously in the recordings. The adequate stimulus is unknown. As local application of smoke is effective only distal to S 20, the effect seems to be mediated by porous sensilla. These hairs are distributed on S 1–19 (Igelmund 1987).

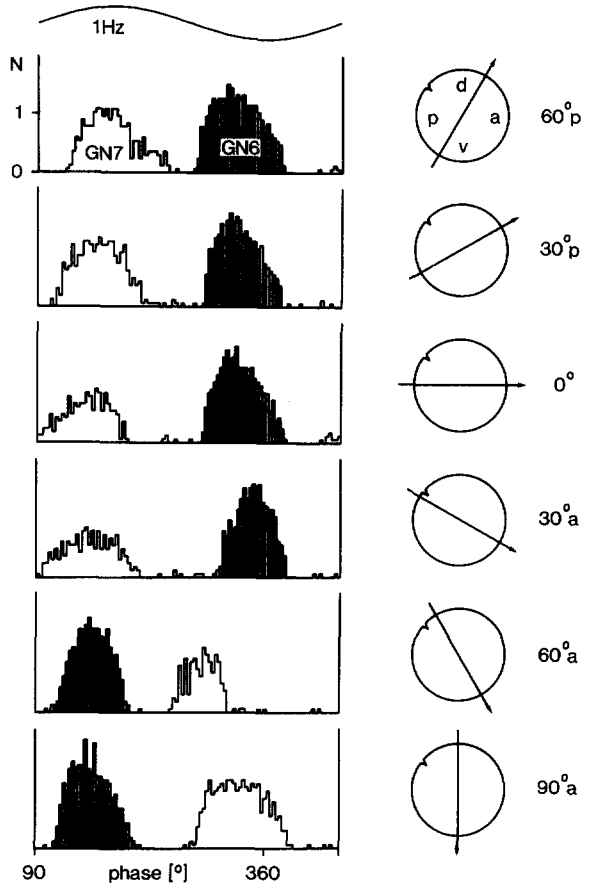

Fig. 7. Distribution of potentials from GN6 and GN7 as a function of the stimulus direction. The stimulus was a sinusoidal deflection at the S 21/22 border with an amplitude of ± 5° about a center position of 0°, a frequency of 1 Hz, and various inclinations of the plane of movement. Twenty periods were analyzed for each situation. For histogram explanation, see Fig. 6. In order to high-light the phase reversal, the responses of GN6 and GN7 are distinguished by showing the individual bars for GN6 and only the outline for GN7. The drawing at the right shows the tarsus in cross section and indicates the direction of the stimulus and the position of the slit sense organ Type I. Arrows: direction of movement during the stimulus phase from 0 (= 360°) to 180°. *Abbreviations: a* anterior, *d* dorsal, *p* posterior, *v* ventral

Other giant neurons in the tarsus

In addition to GN1–GN7, activity was recorded from several other afferent neurons which have different functions. The common features of these neurons are small spike amplitudes (less than 0.2 mV in the proximal regions of the tarsus) and conduction velocities (2–3 m/s in the proximal tarsus) which are lower than those of GN1–GN7. The conduction time for action potentials to travel from the tip of the tarsus to the CNS is estimated to be 80 to 120 ms. Some of these neurons respond to mechanical stimuli: action potentials are elicited by touching the anterior surface of the tarsus distal to segments 32 to 38 – the segments where the tarsus can be actively moved (see below). Touching the rear surface of the tarsus in this region is ineffective. The response is phasic and shows little adaptation to repeated stimulation. Because the spikes can be elicited by touching the cuticle with a needle as well as through movement of the bristles it appears that slit sense organs of Type III mediate this activity. These slit sense organs are localized on the rostral surface of the tarsus. With the surface recording method, discrimination of individual action potentials of these neurons is difficult even with multiple simultaneous recordings and analysis of the conduction velocities. Normally even this does not allow a complete discrimination. The action potentials usually could not be discriminated from the noise in recordings from the tibia and femur; occasionally this was also true for recordings from the tarsus.

Giant neurons in the tibia and femur

In the tibia and femur 6 types of afferent spikes originating in these segments were recorded in addition to the afferent action potentials of the tarsal giant neurons and the efferent potentials of large motoneurons. These discharges were selectively elicited by stimulation of sense organs in these segments.

The largest potentials are generated by two interneurons which are organized like GN1 and GN2 and receive convergent input from the bristles of the tibia and femur, respectively (see Igelmund and Wendler 1991).

Four types of afferent potentials are elicited by stimulating the two trichobothria on segments 4 and 13. Sinusoidal deflections of the trichobothrium on S 13 elicit phase-coupled bursts or, for stimulus frequencies of more than 30 Hz, single spikes generated by two neurons (Fig. 8). The activity of the two neurons is in anti-phase. Both neurons show a phasic response to deflections of the hair from the null position. When the hair is deflected only to one side, then only one of the two neurons is activated. The response depends upon the stimulus direction. The most effective deflection is perpendicular to the long axis of the tarsus. The two types of potentials differ in their amplitudes and conduction velocities. Deflections of the trichobothrium on S 4 evoke equal reactions in two other neurons. The potentials of these neurons are distinguishable from one another as well as from the potentials elicited in S 13 on the basis of their amplitudes and conduction velocities. The potentials are always generated in the segment of the stimulated trichobothrium. The conduction velocities of the potentials are in the range of 6.5–7.5 m/s.

Fig. 8. Activity elicited in response to mechanical stimulation of a trichobothrium. The stimulus (lower trace) was a sinusoidal movement of the trichobothrium on tibial segment 13 with a frequency of 5 Hz and an amplitude of ± 6° perpendicular to the longitudinal axis of the tibia. Upper trace: stimulus dependent activity of 2 neurons recorded from the proximal end of the tibia (S 32)

In contrast to the trichobothria on S 4 and S 13, stimulation of the 5 trichobothria on segments 1 to 3 does not activate giant neurons.

Motoneurons

Because of their segmentation the tarsus and tibia are very flexible throughout their length, but active movement at these secondary segmental boundaries is limited to the distal third of the tarsus. Segments 32 to 38 show the largest active movement; here the tarsus can bend more than 120° in the posterior direction. In the region of segments 18 to 29 bending of about 90° in the ventral direction is possible; a small amount of posterior bending is possible distal to S 20.

As described for *H. longicornis* (Beck et al. 1977), active movements of the tarsus are produced by means of two long tendons (cf. Fig. 3a, b) which insert on the tarsus tip and arise from muscles in the region of the tibia-tarsus joint. Therefore no motor spikes can be recorded in the tarsus. In contrast, recordings from the tibia and femur always contain action potentials from motoneurons. Motor activity is distinguishable from afferent potentials on the basis of conduction direction and spike polarity (see Fig. 10). In addition to the relatively small potentials of several continuously active units, which maintain the tonus of the whip musculature, distally conducting potentials of large amplitude occur in response to mechanical stimulation. These large potentials are always followed by movement if the tarsus or tibia is unrestrained. Action potentials arising from motoneurons responsible for movement at the femur-patella joint are only visible in recordings from the femur; potentials recorded from the tibia are associated with movement of the tibia-tarsus joint and the tendons moving the tarsus.

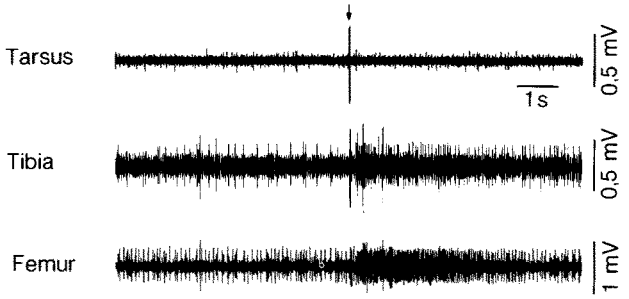

Fig. 9. Motor activity in response to mechanical stimulation of a bristle on the caudal surface of S 60 of the tarsus. Touching bristles on the caudal surface of the tarsus proximal to the actively movable segments leads to an increase in the discharge frequency of tonically active motoneurons in tibia and femur. The arrow indicates stimulus application. The response is not affected by activity in the interneuron GN2; in the example shown here GN2 activity is evident in the recording from the tarsus

Mechanical stimulation of the tarsus at various locations elicits avoidance movements in different directions; these responses are associated with different patterns of motoneuron activity. Touching the caudal surface of the tarsus proximal to the region in which active movement is possible increases the discharge frequency in tonically

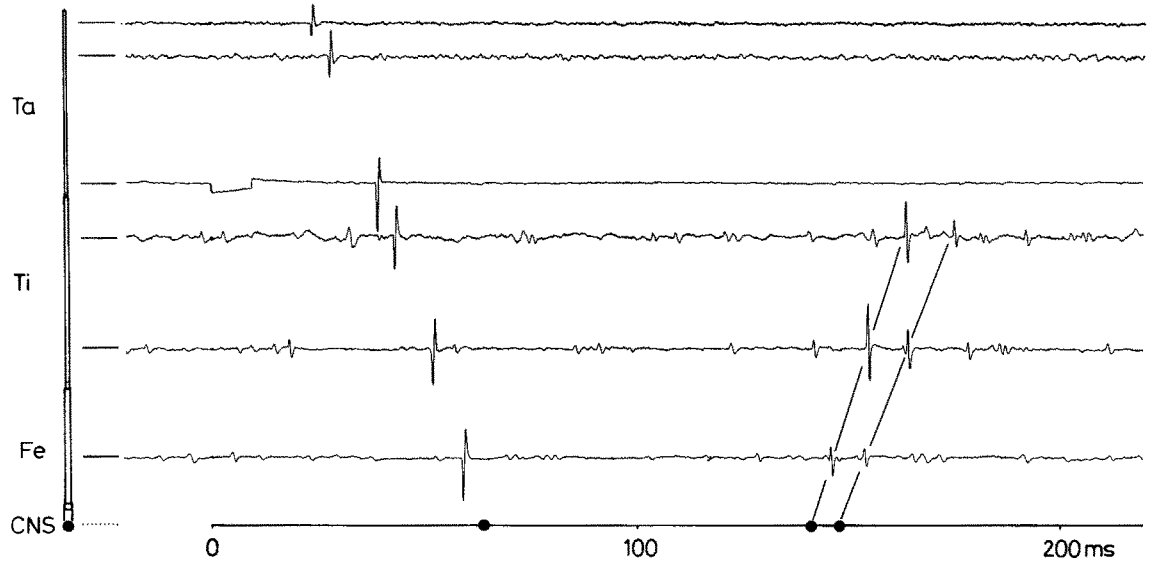

Fig. 10. Motor response to mechanical stimulation of a single bristle on the rostral side of segment 4 of the tarsus. The drawing on the left shows the whip and the recording locations. The traces to the right show simultaneous recordings from 6 sites on the tarsus (Ta), tibia (Ti) and femur (Fe). The spacing between the traces corresponds linearly to the distance between the recording sites. Time zero corresponds to the beginning of a stimulus applied to a bristle on the rostral surface of S 4. After a latency of 24 ms – the latency is unusually long in this example – an action potential elicited in GN1 passes the first electrode. By extrapolation from the conduction time in the femur it is calculated that this potential reaches the CNS within 40 ms. Postulated arrival time marked by a dot on abscissa. Other afferent spikes are not apparent. The stimulus elicits single action potentials in 2 motoneurons. These potentials leave the CNS ca. 141 ms and 149 ms, respectively, after stimulus onset (see dots on abscissa) and are conducted to the tibia (corresponding spikes in the records are connected by lines). The effect of this activity in a freely mobile whip would be a slight flexion of segments 32 to 38 in the tarsus. The motor response is temporally coupled to onset of the stimulus and occurs independently of activity in GN1

active motoneurons of the tibia (Fig. 9) and causes flexing of the tibia-tarsus joint. Touching the rostral surface of the tarsus of the same segments decreases the activity of these motoneurons and causes extension of the tibia-tarsus joint. The extension of this joint is produced hydraulically; it is not associated with activation of motoneurons projecting to the tibia. Touching the rostral surface of the tarsus in the region of S 1 to S 32 elicits the largest motor potentials. These have amplitudes of ca. 1 mV and conduction velocities between 5.5 and 6.5 m/s. This activity is followed by flexion of the tarsus segments 32 to 38. These large potentials are often accompanied by smaller potentials from one or two other motoneurons (Fig. 10). Touching the caudal side of the tarsus distal to S 32 excites smaller motoneurons and leads to an extension of the tarsus at segments 32 to 38.

With repetitive weak stimulation of the rostral tarsus distal to S 32 (as in Fig. 10), the large efferent potentials in the tibial record follow the stimulus with a latency which varies depending upon stimulation and recording positions but is constant under constant experimental conditions. The reflex does not adapt. It does not depend on activation of either of the two giant interneurons, GN1 and GN2. When potentials in the latter occur, there is no change in the temporal relation between stimulus and motor response. The excitation caused by the stimulus is conducted from the distal tarsus to the CNS by neurons the spikes of which often could not be discriminated from the noise (e.g. Fig. 10). Presumably the activity is elicited by stimulation of slit sense organs of Type III.

As shown in Fig. 6, bending the tarsus at the border between segments 21 and 22 causes a much stronger motor response than a similar stimulus at other segments. The former stimulus activates the same motoneurons as does touching of the rostral side of distal tarsus segments.

In behavioural tests of unrestrained animals similar avoidance reactions occurred in response to mechanical stimulation of the whip. Even strong stimuli did not elicit escape responses of the animals.

Discussion

In the light microscopic investigation of the whips the peripheral giant neurons can be distinguished from the mass of smaller sensory cells both by their size and by the structure of their somata. Due to this morphological similarity, they at first appear to be a uniform group. Foelix and Troyer (1980) considered all of the giant neurons they described for *H. longicornis* to be interneurons and thought that the results acquired from some cells could be extended to the others. This study of Foelix and Troyer stimulated the present investigation of *H. elaphus* which provides the first indication of the distinct characteristics of the individual cells. These characteristics are summarized in Table 1.

The individuality of the giant neurons is revealed in their different response spectra as well as in their relationships to the diverse sense organs (bristles, slit sense organs, joint receptors, trichobothria) on one hand and in the amplitude and conduction velocity of the spikes on the other hand. The electrophysiological evidence clearly shows that GN1 and GN2 and the similarly reacting neurons in the tibia and femur are interneurons: action potentials in these neurons are elicited by stimulation of many different bristles and they arise at many different locations (Igelmund and Wendler 1991). Action potentials in GN3, GN4 and GN5 also appear to arise at various locations, so these neurons are probably also interneurons. However, their function is still unknown.

In contrast, the action potentials of GN6 and GN7 as well as those of the tibial neurons reacting on tricho-

Table 1. Characterization of the giant neurons in the whip

Whip segment	Spike type	Spike amplitude (mV)	Conduction velocity (m/s)	Selectively excitable	Sensory modality	Related sensilla	Receptive field	Spike initiation site	Number of neurons	IN or SC	Histol. identified	Soma location
Tarsus	GN1	1.8–2.5	5.5–6.0	+	mechano	ca. 750 bristles	S 1–S 40	S 5–S 40	1	IN	+	S 5
	GN2	0.8–1.6	4.0–4.5	+	mechano	ca. 1500 bristles	S 11–S 72	S 23–S 74	1	IN	+	S 23
	GN3	0.4–0.6	3.5–4.0	−	?	?	?	(prox. S 13)	(1)	(IN)	−	
	GN4	0.3–0.6	3.5–4.0	−	?	?	?	(S 13–S 16)	(1)	(IN)	−	
	GN5	0.2–0.4	3.3–3.9	+	(chemo)	(porous hairs)	S 1–ca. S 20	(distal S 21)	1	(IN)	−	
	GN6	0.2–0.4	3.3–3.7	+	mechano	SSO I or JR	S 21/22	S 22	1	SC	(+)	S 22
	GN7	0.2–0.3	2.8–3.4	+	mechano	JR or SSO I	S 21/22	S 22	1	SC	(+)	S 22
	*	<0.2	2.0–3.0	(+)	mech/ chemo	SSO III, ?	S 1–S 72	S 1–S 74	?	?	−	
Tibia	*			+	mechano	tibial bristles	TiS 1–TiS 32		1	IN	−	
	*			+	mechano	trichobothrium	TiS 4		2	(SC)	−	
	*			+	mechano	trichobothrium	TiS 13		2	(SC)	−	
Femur	*			+	mechano	femural bristles	femur		1	IN	−	

According to the text, the spike types are designated GN1 to GN7. Neurons described in the text without names are marked as "*" in the column "spike type". For spike amplitudes and conduction velocities the ranges of values measured at the proximal end of the tarsus in different animals are given. Abbreviations: IN interneuron, JR joint receptor, S tarsal segment, SC sensory cell, SSO I slit sense organ Type I, SSO III slit sense organ Type III, TiS tibial segment

bothrium deflection always originate at the same place and can only be elicited by stimuli at a specific location. With the recording method used here it cannot be ascertained whether these cells are interneurons or receptor cells. The single spike initiation site and the absence of any convergence suggest that the recorded potentials belong to sensory cells.

A classification of GN1 and GN2 on one side and GN6 and GN7 on the other side into interneurons and primary sensory cells, respectively, is allowed by the combined histological and electrophysiological results. As Fig. 5 shows, not all potentials can be recorded at all positions in the whip. The transition from presence to absence of the spikes is so abrupt that it can not be explained on the basis of a gradual reduction in the axon diameter. Therefore we assumed that the soma of the neuron and the origin of the axon lie in the most distal segment at which the corresponding potentials can be recorded. Because the soma positions postulated on this basis could not be reconciled with the histological findings from *H. longicornis* (Foelix and Troyer 1980), two whips from *H. elaphus* were investigated histologically. The results confirmed the soma positions hypothesized based on the physiological results. This correlation between electrophysiological and histological results holds for GN1, GN2, GN6 and GN7. The neurons GN3, GN4, GN5, as well as the tibial and femural giant neurons have not been investigated histologically.

GN1 and GN2: giant interneurons

These two neurons are the subject of a separate report (Igelmund and Wendler 1991) and therefore will not be further discussed here.

GN6 and GN7: primary sensory neurons

The histological results show that neurons GN6 and GN7, which produce action potentials arising in S 22, can only be primary sensory neurons: 4 large somata belonging to primary sensory neurons are found at this location and no giant somata belonging to interneurons are present. Two of the 4 sensory neurons belong to the large slit sense organ in the segment. The two other sensory neurons are considered to be joint receptor cells. Since only two types of potentials arise in S 22, it is unclear which of the 4 giant sensory cells and therefore which sense organs are responsible.

We hoped to get evidence about the nature of GN6 and GN7 by investigation of their directional specificity, but the unusual structure of the segment border renders the interpretation difficult. In contrast to all the other tarsal segment borders the border S 21/22 is not circular but rather the dorsal margin of S 22 extends into S 21 (see Fig. 2b). The axis of rotation for dorso-ventral movements does not pass through the center of the tarsus but rather through the dorsal margin. As a result, the putative joint receptor, lying caudally in the tarsus, should be maximally stimulated by flexion of S 21 in anterior-dorsal directions. This expected directional specifity is in accord with the activity of GN7.

On the other hand, both neurons GN6 and GN7 reverse the phase of their response to sinusoidal stimulation in a movement plane which coincides with the line from the center of the tarsus through the slit sense organ. Hence, both neurons could belong to the slit sense organ. The adequate stimulus for slit sense organs is compression perpendicular to the long axis of the slit (Barth 1972a, b). As the complicated structure of the segment border does not allow a prediction of the stress vectors in the neighborhood of the slit sense organ, an exact expectation of the directional sensitivity of the slit sense organ on the basis of the morphology cannot be given. Although slit sense organs generally contain two sensory neurons, to date only the activity of one neuron can be found in recordings (Barth 1985). The only known exception are the "campaniform" slit sense organs of opilionids (Dumpert and Gnatzy, cited in Gnatzy 1982).

For a clear decision about the nature of GN6 and GN7 ablation experiments would be necessary. A problem for such experiments lies in the fact that the dendritic terminals of the joint receptor cells are located less than 50 μm away from those of the slit sense organ cells. Hence, destruction of one organ easily damages the other. One possible way could be the selective destruction of the coupling cylinder of the slit sense organ by laser light. Because of the low number of animals and the high risk of autotomy ablation experiments were not performed in the present study.

GN3, GN4, GN5: neurons of unknown function

The function of neurons GN3, GN4 and GN5 is completely unclear. On one hand, a role as mechanoreceptors can be excluded because these neurons do not respond to a wide variety of mechanical stimuli. On the other hand, the neurons could not be stimulated neither with thermal nor with chemical stimuli – with the exception of tobacco smoke which was effective for GN5.

It is evident, in contrast, that with receptors on the whip tarsus the animals actually smell vapors of alcohols, short-chained organic acids, ether, and chloroform as well as complex odors such as that of rotting meat: they release a motor response which leads to a slow withdrawal of the tarsus. However, this information is not transmitted to the CNS through the giant fibers investigated here.

Motor activity

The motor activity recorded from tibia and femur can be divided into tonic activity, which participates in the posture control of the whip, and phasic activity, which is correlated with movements. The animals tend to hold their whips free of contact with their surroundings. Hence, tonic activity is continuously present. Whenever a whip actively or passively gets mechanical contact to an object, an immediate avoidance reflex is evoked which restores the free posture of the whip. No resistance re-

flexes have been observed in the whip. The sense organs responsible for the avoidance reflexes presumably are the different slit sense organs.

Touching of the whip always causes slow avoidance movements of the whip but never an escape response of the whole animal. In contrast, escape behaviour of the whole animal can easily be elicited by mechanical stimulation of the trichobothria on the walking legs. Stimulation of the trichobothria on the whips or stimulation of other whip receptors is ineffective.

Thus, the giant neurons in the whips of whip spiders cannot be accounted for as an escape system analogous to the central giant fiber systems known from other arthropods like cockroaches, crickets and crayfish.

Acknowledgements. We thank Dr. A. Ampuera and Señor Carlitos (IVITA, Pucallpa, Peru), Dr. R.F. Foelix (Fribourg, Switzerland) as well as A. Moegle and G. Rohleder (Berlin) for help in acquiring living whip spiders. Thanks are also due to M.E. Grosmann, H.P. Bollhagen and M. Scheid (Köln) for technical help, to Drs. W. Weber (Köln) and R.F. Foelix for valuable discussions, and to Dr. J. Dean (Bielefeld) for the translation of the manuscript.

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