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FUNCTIONAL SPECIALIZATION OF THE SCOLOPARIA OF THE FEMORAL CHORDOTONAL ORGAN IN STICK INSECTS

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Summary

The femoral chordotonal organ (fCO), one of the largest proprioceptive sense organs in the leg of the stick insect, is important for the control of the femur–tibia joint during standing and walking. It consists of a ventral scoloparium with about 80 sensory cells and a dorsal scoloparium with about 420 sensory cells. The present study examines the function of these scoloparia in the femur–tibia control loop. Both scoloparia were stimulated independently and the responses in the extensor tibiae motoneurones were recorded extra- and intracellularly.

The ventral scoloparium, which is the smaller of the two, functions as the transducer of the femur–tibia control loop. Its sensory cells can generate the known resistance reflexes. The dorsal scoloparium serves no function in the femur–tibia control loop and its stimulation elicited no or only minor reactions in the extensor motoneurones. A comparison with other insect leg proprioceptors shows that a morphological subdivision of these organs often indicates a functional specialization.

Introduction

The femoral chordotonal organ (fCO) is one of the largest proprioceptive sense organs in Orthoptera. It functions as transducer for the femur–tibia (FT) angle. Its anatomy allows easy stimulation and it has been the subject of numerous studies in locusts and stick insects.

The morphology and ultrastructure of the fCO is described in the locust (middle leg: Field and Pflüger, 1989; hind leg: Matheson, 1990; Matheson and Field, 1990; Field, 1991; Shelton *et al.* 1992) and in the stick insect (Füller and Ernst, 1973). In the middle leg of the locust and in all legs of the stick insect, the fCO consists of two scoloparia attached to the FT joint by a common receptor apodeme. In the middle leg of the locust, this anatomical division into two scoloparia is obvious, as they insert at different points on the cuticle and are innervated by different nerve branches. By ablation of one scoloparium and stimulation of the other, Field and Pflüger (1989) have shown that they serve different functions. The smaller, distal scoloparium elicits the known resistance

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reflexes in the extensor and flexor tibiae motoneurones (e.g. Field and Burrows, 1982; Burrows, 1987; Zill, 1987). The proximal scoloparium has no detectable function in the FT control loop but may be involved in vibration reception (Field and Pflüger, 1989).

For the stick insect, a detailed analysis of the response characteristics of sensory cells of the fCO was published by Hoffmann *et al.* (1985) and Hoffmann and Koch (1985), and extensive information has been accumulated about processing of information delivered by the fCO: (i) in the inactive animal (Bässler, 1972*a*,*b*, 1983; Büschges, 1989, 1990; Kittmann, 1991), (ii) in the active animal and during active movements of a single leg (Bässler, 1974, 1988; Bässler and Büschges, 1990; Weiland and Koch, 1987) and (iii) during walking (Bässler, 1983; Cruse and Schmitz, 1983; Schmitz, 1985). In all of these investigations, data were obtained by stimulating the whole organ, disregarding its division into two distinct cords.

In the present study we have investigated the functional specialization of the two scoloparia of the fCO in stick insects. The results obtained will have a strong impact on further investigation of information processing in the control system of the FT joint in the active and inactive animal.

Materials and methods

Preparation

Adult female stick insects, *Carausius morosus* Br., were taken from the culture at the University of Bielefeld. The animal was mounted ventral side up with the coxa and femur of the right middle leg perpendicular to the body axis and fixed with dental cement (Scutan). The tibia was free to move. To expose the femoral chordotonal organ (fCO) and the nerves of extensor and flexor tibiae muscles (nerve F2 and nervus cruris), the cuticle of the femur was cut open on the anterior-ventral side and the flexor tibiae muscle was pinned ventrally.

At FT angles of 20–150 °, the two scoloparia of the chordotonal organ are parallel and it is difficult to identify them by shape or position as they have a common point of insertion on the cuticle and share a common sensory nerve. During relaxation of the fCO (leg extension), the dorsal scoloparium becomes thicker and it is fully relaxed at FT angles beyond 150 °, while the ventral scoloparium remains thin and is still under tension even when the tibia is fully extended.

Two servo-controlled penmotors (Galvanometer Scanner G 300 PD, General Scanning Inc.) were used to move the two cords of the fCO independently (Fig. 1). With the tibia held at an angle of 90 °, the receptor apodeme (RA) connecting the fCO to the tibia was clamped in the forceps of the distal penmotor and cut at the FT joint. Ink particles were applied to both scoloparia at an fCO elongation corresponding to an FT angle of 90 ° and their positions were marked under the dissecting microscope. Relaxation by 500 μm (100 °) using the distal penmotor caused full relaxation and separation of the two scoloparia. One was cut free at its distal end from the receptor apodeme (RA) and clamped into the forceps of the proximal penmotor (Fig. 1). To exclude possible damage artefacts caused by this operation, the ventral scoloparium was cut from the RA in half of the experiments and the dorsal scoloparium in the other half. Results were similar in both

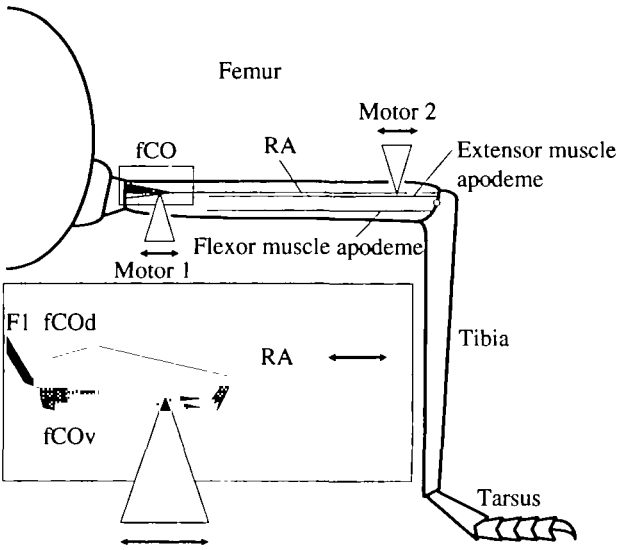

Fig. 1. Schematic drawing of the apparatus used for independent stimulation of the two scoloparia of the femoral chordotonal organ (fCO). A left middle leg is depicted from the anterior. In the situation shown here the larger dorsal scoloparium (fCOd) is stimulated *via* the receptor apodeme (RA) which is attached to motor 2. The RA is cut free from the FT joint distal to the motor. The ventral scoloparium (fCOv) is cut from the RA (see inset) and is stimulated by motor 1. In half of the experiments the stimulus arrangement was as shown here; in the other half, fCOd was cut and stimulated directly and fCOv was left connected to the RA. An elongation movement applied to the fCO mimics flexion whereas a relaxation movement mimics an extension of the FT joint. The locations of the extensor and flexor tibiae muscles are indicated by their muscle apodemes only. Fl, sensory nerve Fl.

cases. Both scoloparia were then extended to their original positions marked by the ink particles (corresponding to an FT angle of 90°) by moving the penmotor forceps. This preparation allowed recording of the response to independent stimulation of either scoloparium, under conditions of normal motoneuronal activity, not changed by artificial relaxation or ablation of one of the scoloparia (c.f. Field and Pflüger, 1989). In the same animal, stimulus programs could be applied to fCOv and fCOd consecutively, a prerequisite for quantitative comparison of the feedback responses. Additional preparation during an experiment, which could disturb the animal and cause changes of gain of the FT feedback system (Kittmann, 1991), were not necessary to alter the scoloparium being stimulated. Even possible interactions between the information coming from both scoloparia could be measured by simultaneously stimulating them with different or identical stimuli. During stimulation of one scoloparium no movement of the other scoloparium was visible even at high magnification of the dissecting microscope. As a control for vibrations possibly transmitted mechanically from one penmotor to the other *via* the apparatus, high-acceleration movements were performed by one of the penmotors with the forceps near, but not connected to, a scoloparium while the other penmotor was connected to fCOv or fCOd. No reactions elicited by these stimuli were observed in these control experiments.

Stimulus programs

Stimulus functions for the two penmotors were taken from two independent function generators (Wavetek 148A). All experiments were carried out with a stimulus amplitude of 300 μm, simulating a tibia movement from the 60° to the 120° position. Sinusoidal stimuli from 0.01 to 20 Hz and ramp-and-hold stimuli with ramp durations of 100, 10 and 6 ms (i.e. 600, 6000 and 10 000 $^\circ s^{-1}$, respectively) and hold times of 1 or 10 s were tested. The two highest ramp velocities were used although during normal walking only velocities up to 2000 $^\circ s^{-1}$ were observed. However, previous studies of the physiology of the afferent neurones (Hoffmann *et al.* 1985) and of local interneurones (Büschges, 1989, 1990) showed that even higher stimulus velocities are perceived and processed. For each stimulus, the responses to 5–40 sinewave cycles or ramps were measured. To prevent habituation, stimuli were separated by pauses of 2 min.

The two penmotors were moved independently. In some experiments, the whole stimulus program was carried out first with one then with the other penmotor. In other experiments, stimuli were tested alternately on the two scoloparia. During stimulation of one scoloparium, the other was held at a position corresponding to the 90° position of the FT joint. Responses to simultaneous stimulation with both penmotors using identical or different stimulus functions, frequencies and phase relationships were measured. Additional information on the different stimulus programs is given in the Results.

Recording techniques

Activity of the extensor tibiae motoneurones was recorded from the extensor tibiae nerve (F2) with an oil-and-hook electrode. Action potentials of the slow extensor tibiae neurone (SETi), the fast extensor tibiae neurone (FETi) and the common inhibitor motoneurone (CI) could be distinguished by their amplitudes. In four animals, flexor tibiae motoneurones were recorded from branches of the main leg nerve (nervus cruris). To expose the mesothoracic ganglion for intracellular recordings from the FETi soma, the sternal plate of the meso- and metathorax was removed. The fatty sheath of the ganglion was cut open and pinned with cactus spines to a wax-coated steel platform positioned under the ganglion. Illumination from the side with a light fibre facilitated location of the cell body. Recordings were carried out with microelectrodes filled with 3 mol l^{-1} KCl (40 MΩ). The FETi neurone was identified by recording from the extensor nerve and the soma simultaneously. Stable recording for up to 3 h was possible.

Data acquisition and evaluation

The stimulus trigger signals, the position signals of the two penmotors and the extra- and intracellular recordings were stored on an FM tape recorder (Racal, Store 7). Statistical analysis was carried out with the aid of two computer systems (Data General DG 20; Apple IIe). For the analysis of the ramp response, the action potentials of the excitatory extensor tibiae motoneurones were separated by a window discriminator, converted into TTL pulses and fed into two channels of a digital input interface. These data together with pretrigger signals (1 s before the start of the elongation ramp) were stored for off-line evaluation. The data were processed to obtain averaged peri-stimulus

time histograms (PSTH) for each motoneurone. The bin width of the PSTH was 200 ms. Data obtained from the intracellular recordings of the FETi motoneurone were digitized at 5 kHz and stored together with the trigger signal (start of the elongation ramp) on disk and averaged off-line. The averages show mean membrane potential ± standard deviation for 512 bins of 2.9 ms each. In soma recordings, the FETi spikes are generally of small amplitude and can therefore be neglected when averaged. The values given for the response amplitude were obtained from recordings where we could clearly distinguish between postsynaptic potentials (PSPs) and spikes.

The spontaneously active SETi neurone is especially suited for statistical analysis of the response to sinusoidal stimuli since even minor input can be detected by its influence on spontaneous activity. The averaged PSTHs (100 bins per stimulus cycle, bin width depending on stimulus frequency; see Figs 4, 7) start at the beginning of the elongation stimulus. The amplitudes of the mean activity (0 harmonic) and the fundamental (first harmonic) as well as the phase relationship between the fundamental and the stimulus were calculated from these PSTHs using discrete Fourier analysis. Significance of modulation was tested using circular statistics (Rayleigh coefficient r, Batschelet, 1965). The phase values of the fundamental reflect the phase of maximum SETi activity in relation to the maximum of fCO elongation. Data obtained from intracellular recordings of the FETi motoneurone were digitized at 1–20 kHz, depending on stimulus frequency, and stored together with the trigger signal (beginning of elongation) on disk for off-line averaging.

Results

Rampwise stimulation

Ramp-and-hold stimuli with an amplitude of 300 μm, ramp durations of 6, 10 and 100 ms, and a hold-time of 10 s were tested. Each stimulus began with an elongation of the appropriate scoloparium from a position corresponding to an FT angle of 120° to a position corresponding to 60° and, after the hold part of the stimulus, a relaxation back to the 120° position. While one scoloparium was stimulated the other was held at a position corresponding to an FT angle of 90°.

SETi neurone activity

Rampwise elongation of the ventral scoloparium of the femoral chordotonal organ (fCOv) caused a rapid increase in the discharge rate of the SETi neurone followed by an exponential decrease to a new tonic value (Fig. 2A,C,E). For slow elongation ramps with durations of 100 ms (Fig. 2A,C), the maximum amplitude of the feedback response, calculated as the difference between the pre-stimulus activity and the peak activity induced by the ramp, was 75.2±14.4 spikes s^{-1} (mean±S.D., N=30, 11 animals). During the hold part of the stimulus, the activity decreased exponentially with time constants between 0.2 and 0.9 s to an increased, tonic activity of 16.8±9.9 spikes s^{-1} (N=30) (the pre-stimulus activity was on average 3.5 spikes s^{-1}). For shorter ramp durations, the maximum amplitudes were lower (about 60 spikes s^{-1}). No significant differences dependent on ramp durations were found for the tonic firing frequency measured 10 s

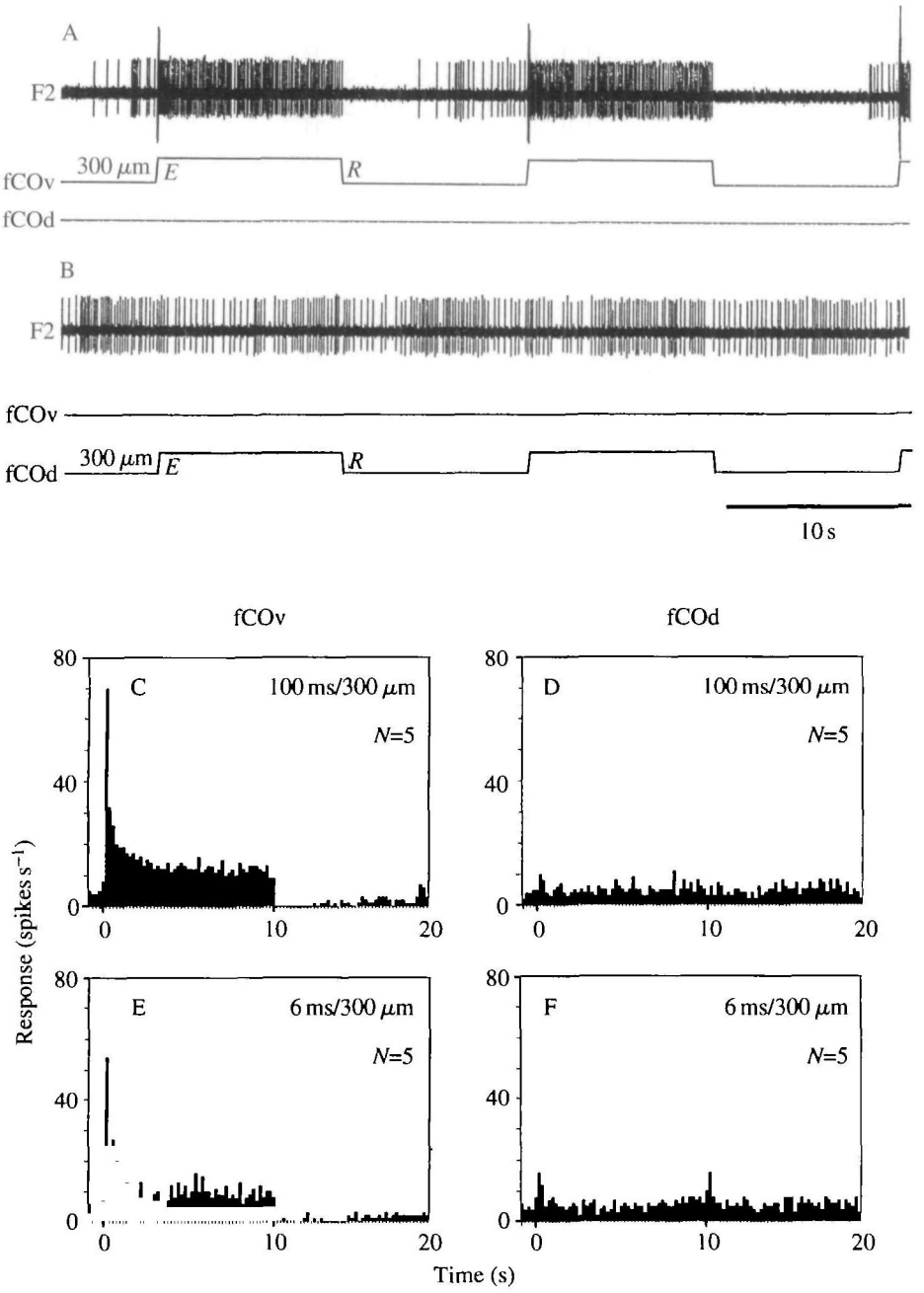

Fig. 2

after the elongation stimulus (χ^2-test, $P>0.1$). Examples from one animal are shown in Fig. 2C,E.

Relaxation of the scoloparium inhibits the tonic activity of the SETi neurone (Fig. 2A,C,E). The extent of this inhibition varied in different animals. For some

Fig. 2. Responses of the extensor tibiae motoneurones of one animal to rampwise stimulation of fCOv (A,C,E) and fCOd (B,D,F). Extracellular recordings (A) from the extensor tibiae nerve (F2) illustrate the excitation of the SETi and FETi motoneurones (medium and large spike amplitudes) during elongation (*E*) and inhibition of SETi spontaneous activity during relaxation (*R*) stimuli applied to fCOv (ramp duration 100 ms). Stimulation of fCOd (B) does not influence the activity of these motoneurones. The slow rhythmic modulation of SETi activity (period duration approximately 10 s) was correlated with ventilation. The averaged responses (to five consecutive ramps of one animal, bin width 200 ms) of the SETi motoneurone to rampwise stimulation with different ramp durations are shown as PST histograms (C–F) in which the beginning of the elongation or relaxation ramp corresponds to 0 s and 10 s, respectively. Stimulation of fCOv always elicits strong reflex responses, but only stimulation of fCOd by the fastest ramp duration (6 ms, F) elicits a weak, significant excitation.

relaxation stimuli, the SETi activity only decreased, while for others, it ceased completely for several seconds. During the hold time of the stimulus, the inhibition declined and SETi activity reached a low tonic value independent of ramp duration. Its mean frequency was 3.5±3.4 spikes s^{-1} (N=65). In some animals, a short excitation of up to four SETi spikes occurred within the first 200 ms after the relaxation stimulus with the shortest ramp duration (6 ms). Similar 'assisting components' have been described for whole fCO stimulation by Bässler *et al.* (1986). This was followed by the inhibition described above.

Rampwise elongation of the dorsal scoloparium of the femoral chordotonal organ (fCOd) did not usually cause significant changes in the SETi motoneurone's activity. The mean activity during stimulation remained at 8.4±3.9 spikes s^{-1} (N=70), a value due mainly to the position of the fCOv, which corresponded to an FT angle of 90 °. However, fast ramps (6 and 10 ms) applied to the fCOd often caused short excitations during the first 200 ms after elongation or relaxation. They were more prominent for the fastest ramps (6 ms, Fig. 2F) where up to five additional spikes were elicited within this interval. During the hold parts of these stimuli there were no significant changes in SETi activity compared with the prestimulus spontaneous activity (χ^2-test, P>0.1).

FETi neurone activity

The FETi motoneurone is not spontaneously active and its excitability shows much variation among individual animals. In nine animals, the FETi motoneurone was excited by fCO stimulation, whereas in two animals, only tactile stimulation at the head or abdomen elicited FETi spikes. These animals were not considered in the quantitative evaluation of FETi activity.

Rampwise elongation of fCOv caused a short burst of FETi spikes (Fig. 2A). For all three ramp durations, this activation was confined to the first 200 ms interval following the start of the ramp. The mean activity measured in this interval was 13.1±9.7 spikes s^{-1} (N=60). In seven animals, rampwise relaxation of the fCOv did not excite the FETi neurone. Fast relaxation ramps (6 ms) sometimes elicited one or two FETi spikes in only two animals. Ramp-and-hold stimuli applied to the fCOd never elicited FETi spikes.

To investigate subthreshold input from the fCO to the FETi neurone, intracellular recordings were made from the soma in six animals in which FETi spikes were elicited by

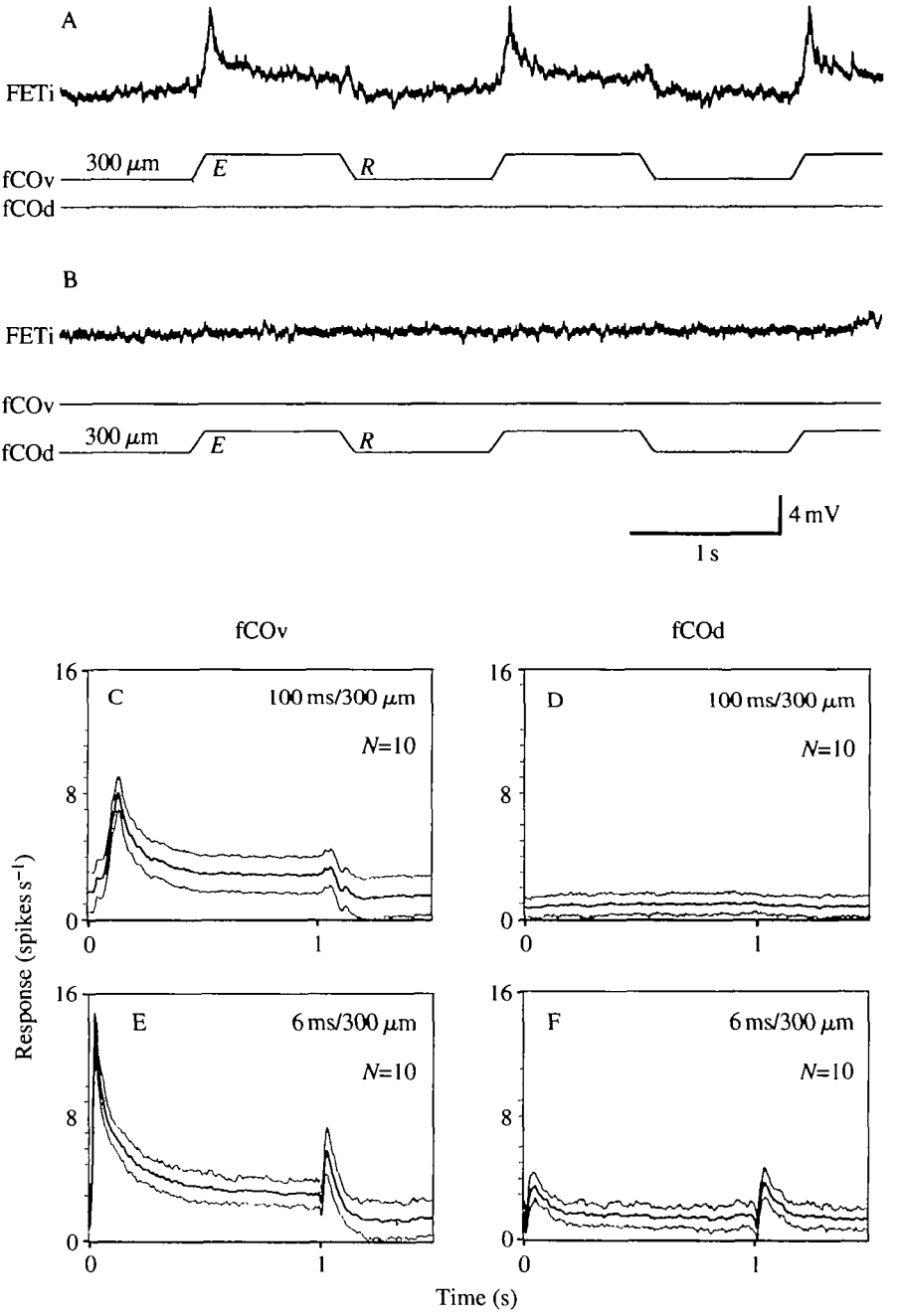

Fig. 3. Intracellular recordings showing the time course of the FETi soma potential during rampwise stimulation of fCOv (A,C,E) and fCOd (B,D,F). The original recordings show the responses of the same animal, first stimulated (100 ms/300 μm) by movement of fCOv (A) and then stimulated by fCOd (B). Averaged time courses of the soma potential during rampwise stimulation of fCOv (C,E) and fCOd (D,F) for different ramp durations start with the elongation ramp; relaxation occurred at 1 s. The averages include 512 time classes of 2.9 ms each. For each time class the mean relative membrane potential ± S.D. is plotted.

fCOv stimulation. To reduce experimentation time, the hold time of the ramp-and-hold stimulus was shortened to 1 s. Rampwise elongation of the fCOv (Fig. 3A,C,E) caused rapid depolarisation of the FETi neurone, sufficient to elicit action potentials. The amplitudes of the depolarisations (relative to pre-stimulus levels) averaged 11.4±1.3 mV (N=90) and 6.4±1.1 mV (N=80) for ramps with durations of 6 and 100 ms, respectively. These depolarisations decreased quickly (time constant about 100 ms). Relaxation stimuli also caused a short depolarisation followed by a weak hyperpolarisation of about 0.5 mV which then decreased quickly. The amplitude of the transient depolarisation increased with stimulus velocity and reached 2.9±1.4 mV (N=90) for the fastest ramps (6 ms).

Rampwise stimulation of the fCOd with ramp durations of 100 ms did not change the membrane potential (Fig. 3B,D). Only the fastest ramps (6 ms) caused short depolarisations of 1.7±0.8 mV (N=80) and 2.1±0.9 mV (N=80) for elongation and relaxation stimuli, respectively. Unlike the response caused by fCOv stimuli, no hyperpolarisation was observed.

Sinusoidal stimulation

The response characteristics of the FT control loop cannot be completely described by analyzing the input–output characteristics with a single type of stimulus function (e.g. ramps), as this system shows several nonlinearities (see Bässler, 1983; Kittmann, 1991). We therefore tested the possible contribution of the fCOd to the feedback response when sinusoidal stimuli are applied to it. The frequency–response curves of the extensor tibiae motoneurone's activities were measured for independent stimulation of both parts of the fCO. Each stimulus consisted of 10–30 sinewave cycles of the tested frequency. Because of the long duration, only 3–5 cycles were tested for the lowest frequency (0.01 Hz). During the stimulation of one scoloparium, the other was held in a position corresponding to an FT angle of 90 °.

SETi neurone activity

Sinusoidal stimulation of the fCOv with a stimulus amplitude of 300 μm and stimulus frequencies between 0.01 and 20 Hz caused feedback responses similar to those described for the stimulation of the whole fCO (Bässler, 1983; Kittmann, 1984). The spontaneous activity of the SETi neurone was modulated by the stimulus, increasing during elongation and decreasing during relaxation (Fig. 4A,C). With frequencies up to 10 Hz, all fCOv stimuli (N=75) caused significant SETi activity modulation (Rayleigh test, P<0.01). At the highest stimulus frequency tested (20 Hz), the modulation was significant in six out of nine animals (Rayleigh test, P<0.01).

The mean activity and the amplitude of the fundamental (describing the modulation of spike activity by the stimulus) increased with increasing stimulus frequency (circles in Fig. 5A,B). The mean activity was 10.8±5.8 spikes s^{-1} (N=15) at 0.01 Hz stimulus frequency and increased to 74.3±18.6 spikes s^{-1} (N=330) at 20 Hz. The amplitude of the fundamental increased in parallel from 12.2±5.3 spikes s^{-1} (N=15) at 0.01 Hz to 37.1±8.7 spikes s^{-1} (N=180) at 2 Hz and remained at about 40 spikes s^{-1} at higher stimulus frequencies. The phase frequency plot (Fig. 5B) reflects the mean phase relationships between the fundamental and the maximum of elongation of the fCO

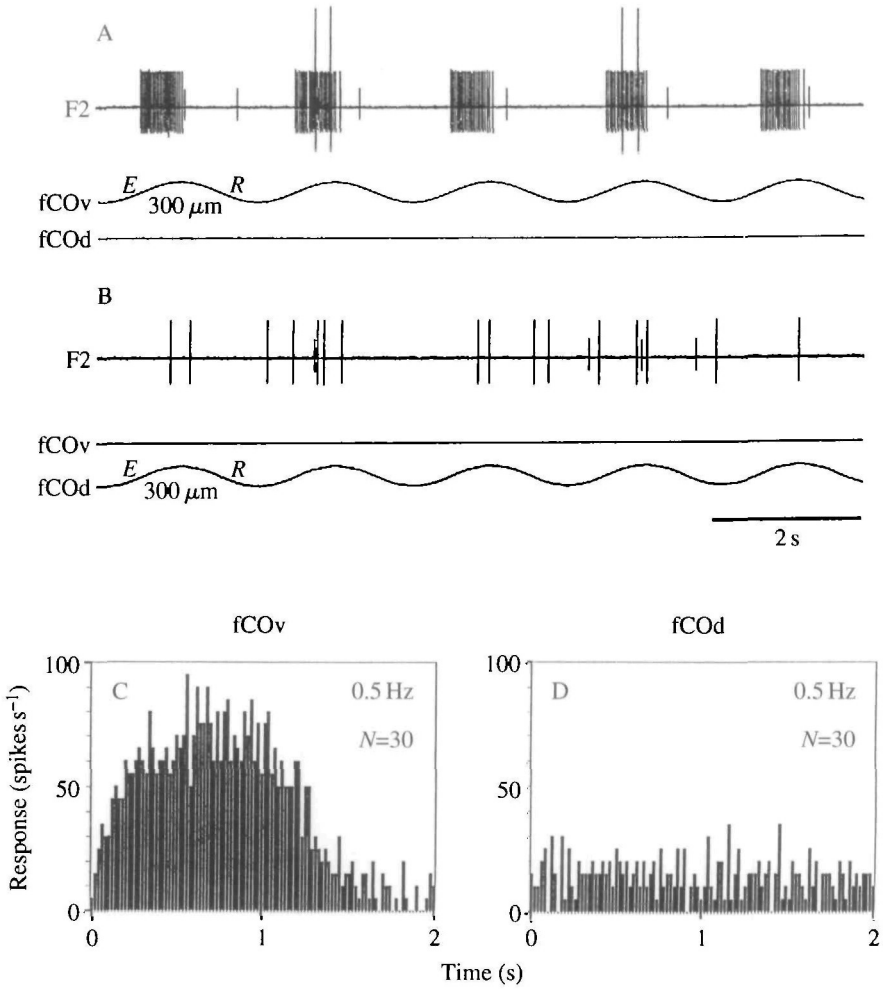

Fig. 4. Responses of the extensor tibiae motoneurones to sinusoidal stimulation of fCOv (A,C) and fCOd (B,D). Spikes of small, medium and large amplitudes correspond to the CI, SETi and FETi motoneurones, respectively. The PST histograms show the SETi response averaged for 30 stimulus cycles, triggered at the beginning of the elongation part of the stimulus. With fCOv stimulation (C) the mean activity of the SETi motoneurone is significantly modulated by the stimulus. This is not the case with fCOd stimulation (D).

stimulus for each stimulus frequency tested. The phase values are positive at stimulus frequencies lower than 5 Hz, indicating that maximum activity leads the maximum elongation of the fCOv. At higher stimulus frequencies, the SETi response lagged behind the stimulus movement.

Stimulation of the fCOd with amplitudes of 300 μm and frequencies between 0.01 and 20 Hz (with the fCOv unstimulated in the 90° position) caused little or no reaction in the extensor motoneurones (Fig. 4B,D). The mean activity of the SETi neurone remained low (between 3.7 and 7.2 spikes s^{-1}) and did not show significant differences from pre-stimulus values (χ^2-test, $P>0.05$). In 56 % of all stimuli tested, no significant modulation

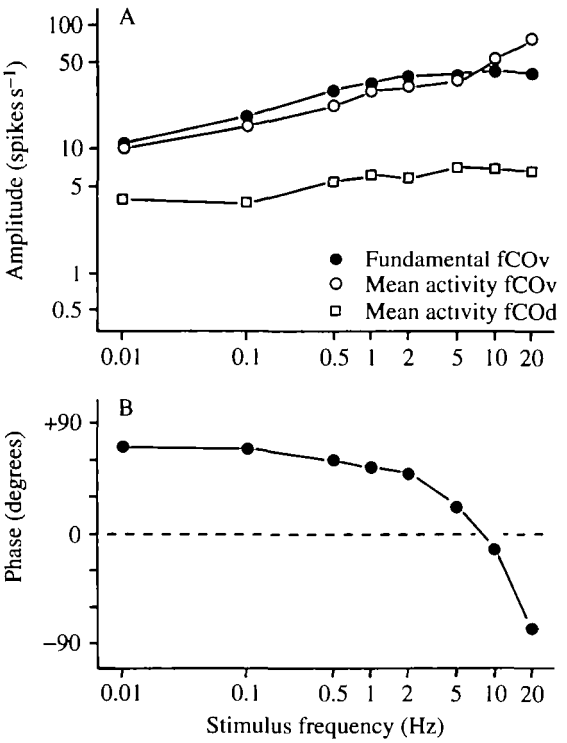

Fig. 5. Amplitude and phase frequency plots of the responses of the SETi motoneurone to sinusoidal stimulation of fCOv (circles). For stimulation of fCOd, modulation of SETi activity was weak, mostly insignificant, and thus only the mean activity (squares) is plotted. The amplitudes of the mean activity (open symbols) and of the fundamentals (filled circles) (in A) and the phase values (in B) are mean values from averaged PST histograms of all animals.

of the mean activity was found (Rayleigh test, $P>0.1$). In the other cases, the amplitude of the fundamental was less than 9 % of the corresponding values for fCOv stimulation and never exceeded 7 spikes s^{-1}. In cases where significant modulations were observed, the phase values were similar to those observed with fCOv stimulation.

FETi neurone activity

Upon stimulation of fCOv, extracellular recordings revealed increased activation of the FETi neurone at higher stimulus frequencies. For stimulus frequencies up to 1 Hz only five of the nine animals showed a weak activation (<5 spikes s^{-1}), but all nine animals showed an excitation at stimulus frequencies between 1 and 20 Hz. The phase relationship between the stimulus and the fundamental was similar to that described for the SETi neurone (Fig. 5B).

Intracellular recordings from the FETi soma showed depolarisation during elongation of the fCOv and hyperpolarisation during relaxation at low stimulus frequencies (Fig. 6A,C). At higher stimulus frequencies, the mean value of the membrane potential showed a shift towards depolarisation during consecutive stimulus cycles. This becomes

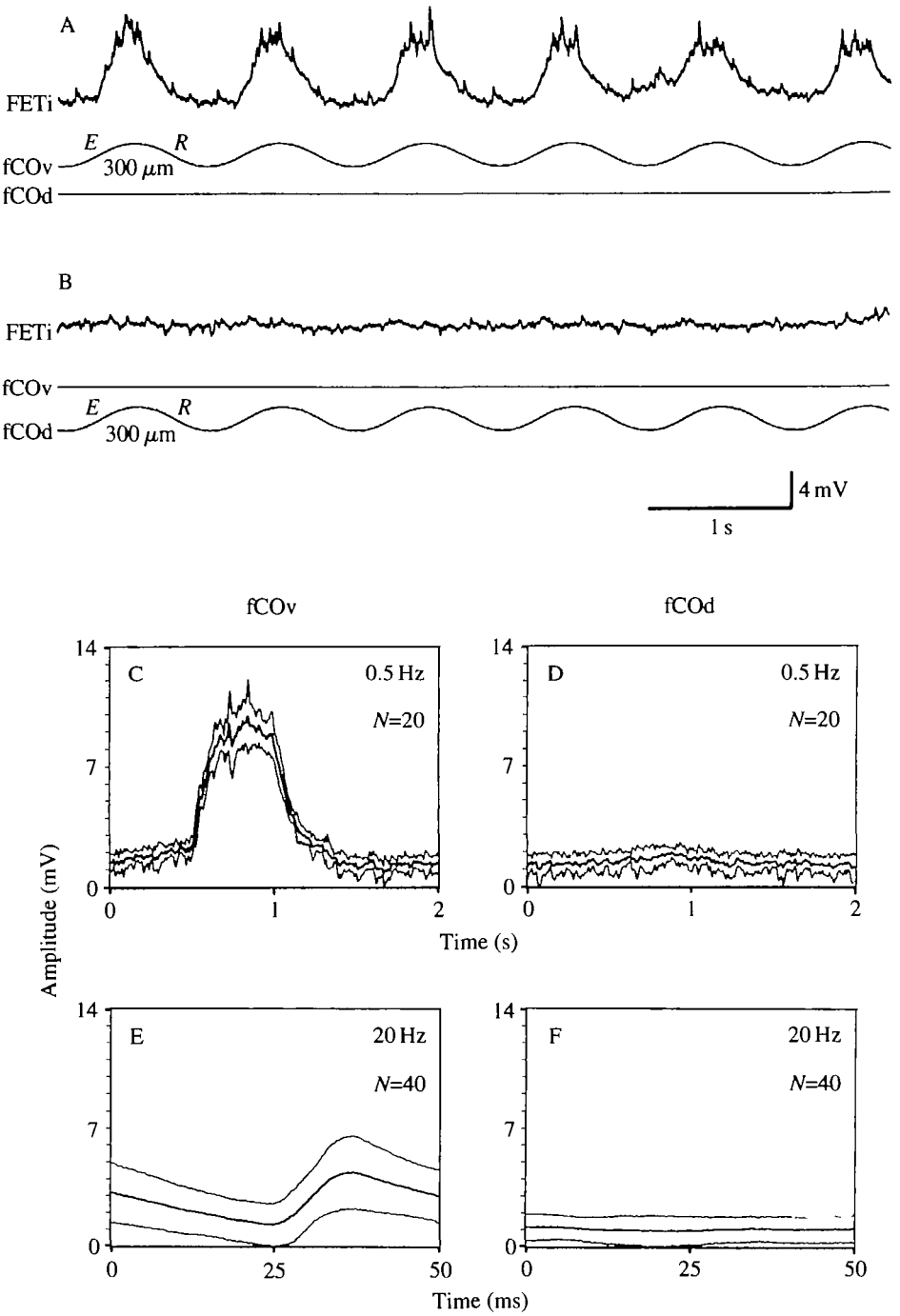
A
FETi
fCOv
E
R
300 μm
fCOd
B
FETi
fCOv
fCOd
E
R
300 μm
4 mV
1 s
fCOv
fCOd
C
0.5 Hz
N=20
D
0.5 Hz
N=20
Amplitude (mV)
Time (s)
E
20 Hz
N=40
F
20 Hz
N=40
Time (ms)

Fig. 6

Fig. 6. Intracellular recordings from the FETi soma showing the time course of membrane potential modulation during sinusoidal stimulation of fCOv (A) and fCOd (B) in the same animal stimulated at 1 Hz. The averaged time courses of the soma potential (C–F) show responses during sinusoidal stimulation with different stimulus frequencies. Sampling was triggered by the beginning of the elongation part of the stimulus. Averages are composed of 512 time classes of 3.91 ms (C,D) and 0.09 ms (E,F) each. For each time class, the mean relative membrane potential ± S.D. is plotted.

apparent from the larger standard deviations in Fig. 6E. Stimulation of the fCOd at frequencies from 0.01 to 20 Hz did not elicit any spikes. Intracellular recordings showed no significant changes of membrane potential correlated with sinusoidal fCOd stimulation (Fig. 6B,D,F).

Simultaneous stimulation of fCOv and fCOd

Under natural conditions, both scoloparia of the fCO are stimulated equally, as they share common points of insertion at the cuticle and receptor apodeme. The information from both scoloparia might thus be processed together, resulting in common output to the motoneurones. During simultaneous stimulation the mean activity of the motoneurones is increased by stimulation of fCOv, which might possibly facilitate the system for input from fCOd. To determine whether this is the case, and whether the weak response to stimulation of the fCOd might be caused by the low spontaneous activity of the motoneurones, simultaneous and separate stimulation of fCOv and fCOd were performed with identical and different stimulus functions.

Simultaneous sinusoidal stimulation of fCOv and fCOd with identical stimulus frequencies

In these experiments, both penmotors were driven by the same function generator (0.5 Hz, 300 μm). Stimulus amplitude, frequency and phase were therefore identical for both parts of the fCO. Mean activity and amplitude of the fundamental were the same as when the fCOv alone was stimulated (t-test, $P>0.1$). The same was true when the phase between the two penmotors was shifted by 180°.

Simultaneous sinusoidal stimulation of fCOv and fCOd with slightly different stimuli

Upon stimulation of both scoloparia with two independent function generators at slightly different frequencies (e.g. fCOv 0.495 Hz, fCOd 0.484 Hz) the phase relationship between the two stimuli changed continuously. After 82.8 s (i.e. 41 and 40 stimulus cycles, respectively) the phase shift completed a full cycle (360°) and both stimuli were again in phase. The response of the SETi neurone within this 82.8 s interval – first to the fCOv stimulus and then to the fCOd stimulus – was evaluated. While a strong modulation was evident with fCOv stimulation (Rayleigh $r=0.44$, $P<0.001$) fCOd stimulation did not cause any significant modulation (Rayleigh $r=0.04$, $P>0.1$, Fig. 7).

Flexor motoneurones

Four animals were examined to investigate which part of the fCO is responsible for the reflex activation of flexor motoneurones recorded during sinusoidal stimulation. In all

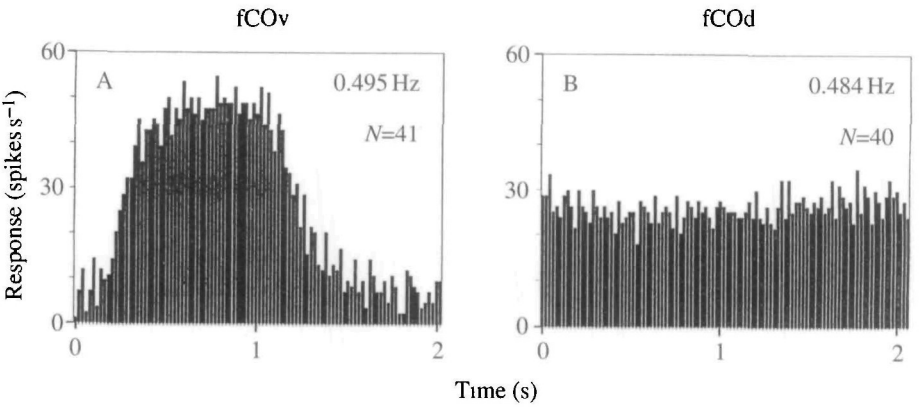

Fig. 7. SETi activity during simultaneous but independent sinusoidal stimulation of the two scoloparia at slightly different stimulus frequencies. The PST histograms are sampled over identical time intervals, but with different trigger points. (A) The SETi response averaged for 41 stimulus cycles triggered at the beginning of the elongation movement applied to fCOv. (B). The PSTH for 40 stimulus cycles triggered at the beginning of the elongation movement applied to fCOd.

animals, flexor motoneurones were excited only during relaxation stimuli applied to the fCOv and no reflex response was observed during stimulation of fCOd.

Discussion

The fCOv is the transducer of the FT control loop

Our results clearly demonstrate a functional specialization of the two scoloparia of the femoral chordotonal organ (fCO) in stick insects. Only the smaller, ventral scoloparium (fCOv) serves as the transducer of the femur–tibia (FT) angle in the FT control loop. Under open-loop conditions, its stimulation elicits the same responses in the extensor and flexor tibiae motoneurones as described for the stimulation of the whole organ with ramps (Bässler, 1983) and sinewave functions (Kittmann, 1984). The larger, dorsal scoloparium (fCOd) has no obvious function in the FT control loop. It did not elicit significant responses in the extensor tibiae motoneurones for most of the stimuli used. In the few cases in which fCOd stimulation with high velocity and acceleration caused weak excitation in the motoneurones it is not clear whether these responses were due to activation of velocity- or acceleration-sensitive cells of the fCOd. As both cords of the fCO share common points of insertion on the cuticle, it is quite conceivable that these fast stimuli excited sensory cells of the fCOv by mechanical coupling through the tissue of the scoloparia.

Impact on further studies of the FT control loop

It is surprising that the only function of the fCO known involves only about 80 sensory cells of the fCOv, although the organ as a whole consists of 500 sensory cells. The restriction of the transducer to a relatively small number of sensory cells encourages further study of neural information processing in the FT control loop. The feedback

system responds to an extremely wide range of inputs, with respect to shape, amplitude, frequency, velocity and acceleration of the signal (Bässler, 1983). Its transducer must detect and distinguish all these stimuli. It is likely, therefore, that, as in the locust mtFCO (Matheson, 1990), the stick insect fCOv includes small subpopulations of sensory cells with distinct physiological properties. Thus, investigation of single sensory cells with different response characteristics and their contribution to the FT feedback response should provide deeper insight into the processing of different stimulus parameters in the central nervous system of insects, especially since the FT control loop of the stick insect is well suited for a quantitative system analysis.

In view of the different functions of the two scoloparia, it is important to determine whether differences exist in the response properties of their sensory cells. This could reveal which of the physiological types of fCO sensory cells described (Hoffmann *et al.* 1985) are present in the fCOv, the feedback transducer that supplies information to the interneurones of the FT control loop (Büschges, 1990; Bässler and Büschges, 1990). A study of response characteristics of sensory cells of the fCOd could help determine the function of this scoloparium. Recent results on the metathoracic fCO in the locust (Field, 1991; Shelton *et al.* 1992) demonstrated the importance of the mechanical structures connecting the sensory neurones to the receptor apodeme. The total relaxation of the dorsal scoloparium at FT angles larger than 150° suggests that this scoloparium only functions at smaller FT angles than this.

The central projections of the fCO terminate in two different areas of neuropile (Schmitz *et al.* 1991; Kittmann *et al.* 1991; c.f. Field and Pflüger, 1989). Further studies on the physiological characteristics of single sensory cells in the two scoloparia and their central projections could lead to a better understanding of the organisation of sensory neuropiles in insects which process different proprioceptive information.

Comparison with other systems

The morphological and functional subdivision of a leg proprioceptor is not unique to the fCO. It is also described in the hairplates that serve as transducers of the thoraco-coxal and coxo-trochanteral feedback loop in stick insects. Both feedback loops show system characteristics similar to those found in the FT control loop. The hairplates consist of two subgroups of mechanoreceptive hairs (Tatar, 1976; Schmitz, 1986*a*,*b*) which are successively bent by the joint membrane when the joint is flexed (Wendler, 1964). In both hairplates, only the group with the smaller number of hairs functions as a transducer of the control loop and can elicit resistance reflexes (Schmitz, 1986*a*,*c*; Büschges and Schmitz, 1991). The larger subgroup may play a role in the intersegmental coordination of leg movement (Dean and Schmitz, 1992).

In grasshoppers, where the morphology of the fCO is known for several species (Moran *et al.* 1975; Slifer and Sekhon, 1975; Field and Rind, 1976; Theophilidis, 1986; Matheson and Field, 1990), morphological division of the two scoloparia of the fCO varies. In the unspecialized walking legs of the locust, the two scoloparia are separated. They are connected to the tibia *via* the same apodeme, but have different points of insertion at the cuticle and are supplied by different nerve branches. In the metathoracic legs of the New Zealand weta, a state of fusion similar to that in the stick insect leg was

found (Field and Rind, 1976). The two cords fuse proximally, where they have a common point of insertion at the cuticle and they share a common nerve.

The fCO of the metathoracic leg of the locust has been the subject of extensive morphological and physiological studies. Its connection with motoneurones and interneurones and its function in local reflexes have been investigated with reference to principles of sensorimotor integration (Field and Burrows, 1982; Burrows, 1987, 1988, 1989; Burrows *et al.* 1988; Field, 1991; Laurent and Burrows, 1988, 1989; Shelton *et al.* 1992; Zill, 1985*a*,*b*). The fCO in these specialized jumping legs differs in several aspects from its homologue in normal walking legs: there is only one cord visible, the number of sensory cells is reduced and it has an additional strand that connects it with the flexor tibiae muscle. Investigations by Matheson and Field (1990) showed two different groups of sensory cells within the metathoracic scoloparium, suggesting that the scoloparia are fused. The maps of cells with larger somata show that fCO neurones exhibiting similar responses have somata in comparable locations within the scoloparium (Matheson, 1990). Sensory neurones within the same scoloparium but with different response characteristics have different central projections and therefore might have different connections with motoneurones and interneurones (Matheson, 1992). Recent results of Field (1991) and Shelton *et al.* (1992) indicate that the response characteristics of sensory cells might be strongly influenced by the mechanical properties of their attachment to the apodeme. However, information about functional aspects of the morphological division is available only for the middle leg of the locust (Field and Pflüger, 1989). These authors found that only the scoloparium with the smaller number of sensory cells serves as a proprioceptive transducer in resistance reflexes. For the other scoloparium, the authors assumed a vibration sensitivity.

The functional subdivision of a sense organ whose different parts receive a common input (e.g. *via* the receptor apodeme) leads to the question of whether neural pathways serving specific functions (e.g. resistance reflex) are already separated at the level of proprioceptive sensory cells. Neuroanatomical studies that revealed two different projection areas of the sensory cells of the two scoloparia in the locust (Field and Pflüger, 1989) and in the stick insect (Schmitz *et al.* 1991) support this concept.

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