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## SHORT COMMUNICATION

# THE PHYSIOLOGY OF SENSORY CELLS IN THE VENTRAL SCOLOPARIUM OF THE STICK INSECT FEMORAL CHORDOTONAL ORGAN

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The leg joints of invertebrates are governed by neural control loops that control their position and velocity during movements (for reviews, see Bässler, 1983, 1993). These neural control loops rely on sensory feedback about the position and velocity of the controlled leg joint. In invertebrates, this sensory feedback is provided by external (e.g. hair fields, hair rows) and/or internal sense organs (e.g. chordotonal organs). The femoral chordotonal organ (fCO) serves as the main proprioceptor in the control loop governing the femur–tibia (FT) joint of the insect leg. The fCO measures the position and movement of this joint (e.g. Bässler, 1965, 1993; Burns, 1974; Usherwood *et al.* 1968; Zill, 1985).

Previous investigations have described the physiology of sensory cells within femoral chordotonal organs (e.g. stick insect, Hofmann *et al.* 1985; Hofmann and Koch, 1985; locust, Matheson, 1990; Matheson and Field, 1990). Numerous investigations have been undertaken into the central processing of sensory information provided by the fCO to gain an insight into the control of FT joint movement during different behavioural tasks, for example during resistance reflexes in the standing animal (locust, Burrows, 1987, 1988; Burrows *et al.* 1988; stick insect, Bässler, 1988; Büschges, 1989, 1990; Driesang and Büschges, 1993) or during active movements (stick insect, Bässler, 1988; Bässler and Büschges, 1990).

Most previous studies have not, however, taken into account the morphological separation of the fCO into two distinct scoloparia in the legs of some species (stick insect, Füller and Ernst, 1973; Hofmann *et al.* 1985; Hofmann and Koch, 1985; locust middle leg, Burns, 1974). It has been inferred that the whole fCO supplies position and velocity information about the FT joint. In contrast, recent studies of leg reflexes have shown that only its smaller scoloparium (Fig. 1A), containing approximately one-sixth of the total number of sensory neurones, provides the sensory information that is used by the FT control loop (locust, Field and Pflüger, 1989; stick insect, Kittmann and Schmitz, 1992). These studies did not show what types of sensory neurones are located in the ventral part of the fCO and thus contribute to the FT control loop. We have therefore investigated the physiology of sensory neurones that are located in the ventral scoloparium of the fCO.

Key words: movement detection, chordotonal organ, joint control, walking system, *Cuniculina impigra.*

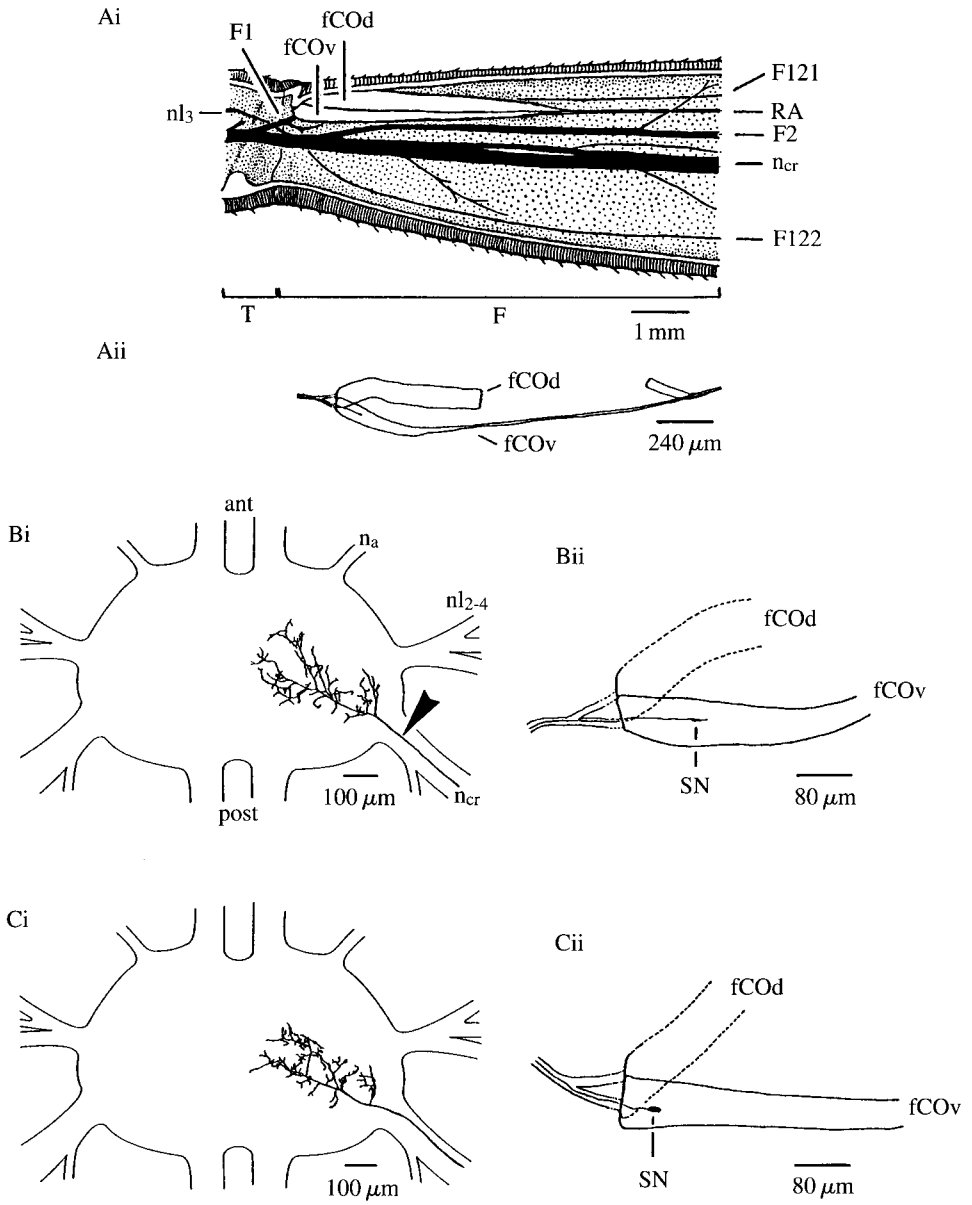


Fig. 1

The physiology of sensory neurones in the ventral scoloparium of the fCO (fCOv) in the stick insect middle leg was investigated on an air-suspended, mechanically isolated table. *Cuniculina impigra* (Br.) from our laboratory culture were mounted dorsal side up on the rim of a foam platform with the mesothoracic and most of the metathoracic segment located in an enclosure (20 mm×60 mm) containing stick insect saline (Weidler and Diecke, 1969). The right middle leg was fixed at right angles to the thorax with dental

Fig. 1. Location and morphology of the femoral chordotonal organ (fCO) in the middle leg of the stick insect. (Ai) Location of the fCO in the femur. T, trochanter; F, femur; fCOd, dorsal scoloparium of the fCO; fCOv, ventral scoloparium of the fCO; $nl_3$, nervus lateralis 3; F1, nerve innervating the fCO; F121, nerve 121; RA, receptor apodeme of the fCO; F2, nerve 2; $n_{cr}$, nervus cruris; F122, nerve 122. (Aii) Preparation showing the two scoloporia of the fCO; the fCOd (cut) and the fCOv (intact). (B) Morphology of a velocity-sensitive neurone in the fCOv. (Bi) Central arborizations of this sensory neurone in the mesothoracic ganglion. (Bii) Location of the soma (SN) in the fCOv. ant, anterior; post, posterior; $n_a$, nervus anterior; $nl_{2–4}$, nervi laterali 2–4. The arrow in i denotes the approximate location of the electrode used to make recordings from fCO afferents. (C) Morphology of an acceleration-sensitive neurone in the fCOv. (Ci) Central arborizations of this sensory neurone in the mesothoracic ganglion. (Cii) Location of the soma in the fCOv.

cement (Protemp, ESPE) with the tibia pointing down at an angle of approximately 130 °. The whole femur was opened, and the tibial extensor, the retractor unguis and the bulk of the tibial flexor muscles were removed so that the femoral chordotonal organ and its apodeme were totally exposed. All nerves in the femur ($n_{cr}$, F121, F122, F2; Bässler, 1983) were cut distal to the fCO. The apodeme of the fCO was fixed at 130 ° in the clamp of a mechanical stimulator (Hofmann *et al.* 1985) and cut distally. The thick dorsal scoloparium of the femoral chordotonal organ was cut, leaving only the thin ventral scoloparium (fCOv) attached to the apodeme (Fig. 1A). Ramp-and-hold stimuli with different position, velocity and acceleration values generated by a wave-form generator (Hofmann *et al.* 1985) were applied to the ventral scoloparium (position values were equivalent to FT joint angles ranging from 170 ° to 30 ° (−400 $\mu$m to +1000 $\mu$m; in *Cuniculina impigra* a 100 $\mu$m change of fCO length corresponds to a change of 10 ° in FT angle (Weiland *et al.* 1986); velocity values ranged from 12.5 degrees $s^{-1}$ (125 $\mu m\,s^{-1}$) to 6700 degrees $s^{-1}$ (67 000 $\mu m\,s^{-1}$) and acceleration values ranged from 30 degrees $s^{-2}$ (3000 $\mu m\,s^{-2}$) to $1.94\times10^7$ degrees $s^{-2}$ ($1.94\times10^9$ $\mu m\,s^{-2}$). The activity of sensory neurones in the fCOv was recorded intracellularly from their axons in the nervus cruris ($n_{cr}$) close to its entrance into the mesothoracic ganglion (Fig. 1B). Other procedures necessary for intracellular recordings have been described previously (Büschges, 1989, 1990). To ensure that only sensory neurones were investigated, all recorded neurones were stained either with Lucifer Yellow or with cobalt chloride according to established procedures (Stewart, 1978; Bacon and Altmann, 1977). After injection of dye into a sensory neurone, the ganglion and, in some preparations, the fCO (see below) were fixed with 4 % paraformaldehyde before being removed from the animal. The tissues were then dehydrated, intensified (for cobalt filled) and cleared according to standard procedures. The projections of the sensory neurones in the mesothoracic ganglion (Fig. 1B) were similar to those described by Schmitz *et al.* (1991) for the projections of the whole fCO. Intracellular recordings were made from 82 sensory neurones in 52 animals. They were described and classified according to the nomenclature used by Hofmann *et al.* (1985) and Hofmann and Koch (1985).

The mean activity of twenty recorded neurones was strongly affected by the position of the fCOv; fourteen of these increased their discharge rate with increasing elongation of the fCOv (P+; the characteristics of five such neurones are shown in Fig. 2A), five neurones showed the opposite dependency (P−; one is shown in Fig. 2A) and one

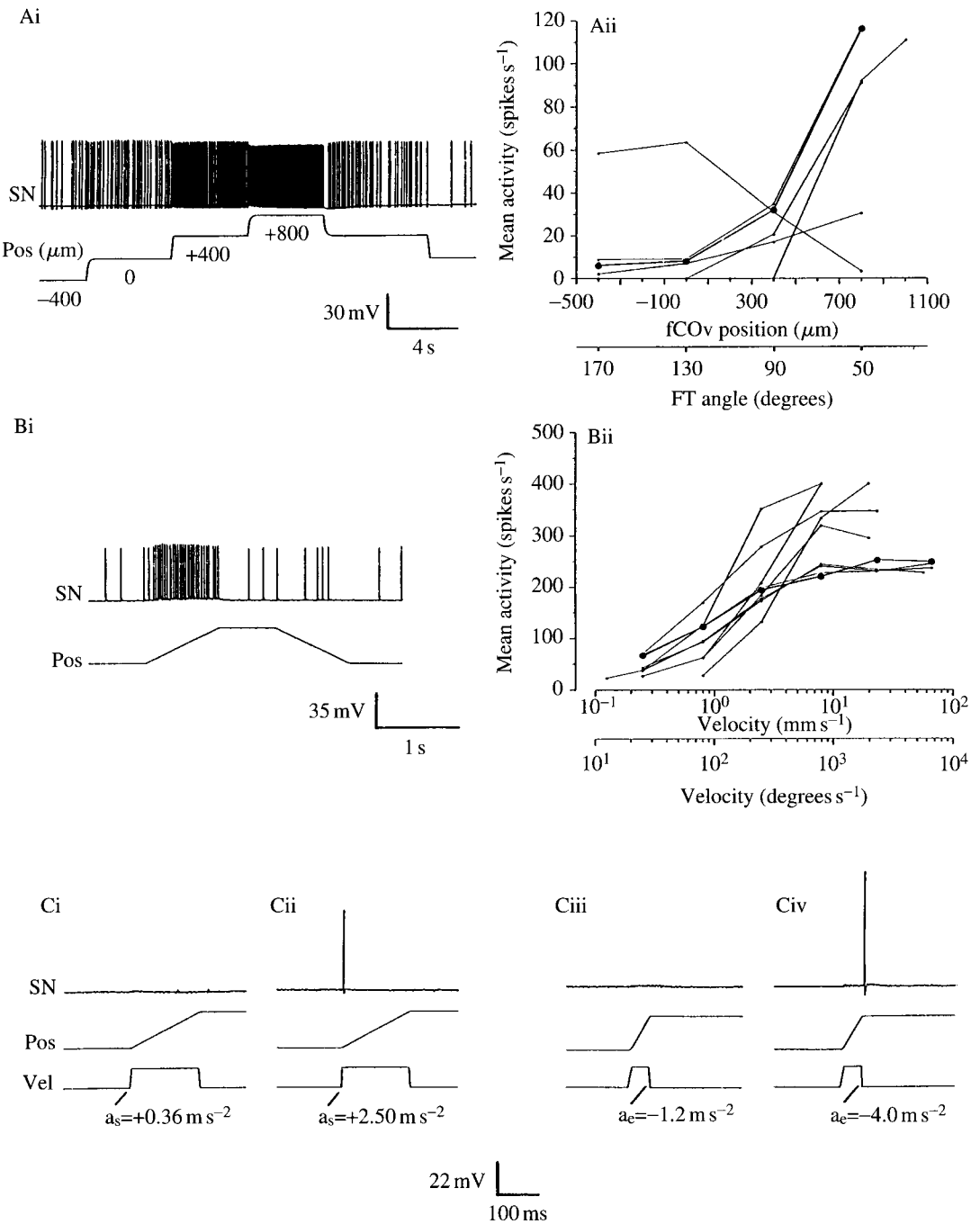
Ai
SN
Pos (μm)
+800
+400
0
−400
30 mV
4 s
Aii
Mean activity (spikes s−1)
120
100
80
60
40
20
0
−500
−100
300
700
1100
fCOv position (μm)
170
130
90
50
FT angle (degrees)
Bi
SN
Pos
35 mV
1 s
Bii
Mean activity (spikes s−1)
500
400
300
200
100
0
10−1
100
101
102
Velocity (mm s−1)
101
102
103
104
Velocity (degrees s−1)
Ci
Cii
Ciii
Civ
SN
Pos
Vel
as=+0.36 m s−2
as=+2.50 m s−2
ae=−1.2 m s−2
ae=−4.0 m s−2
22 mV
100 ms

Fig. 2

Fig. 2. Physiology of sensory neurones in the fCOv. (Ai) Intracellularly recorded activity of a position-sensitive unit (P+, SN) in the fCOv at different positions (Pos) of the fCOv. The relationship between position and firing frequency for this unit is given in Aii (large filled circles). Hysteresis in the activity of this unit is clearly visible at the +400 $\mu$m position: approaching this position from a more relaxed or more elongated position results in different tonic firing rates. (Aii) Characteristics of five position-sensitive units in the fCOv (four P+ units and one P− unit). The mean activity for each unit was calculated as the average value at a given position for 2 s (starting 1 s after the position had been set) in two trials starting at the most relaxed position. Position values are given both in micrometres for fCOv length and as corresponding degrees for FT angle. (B) Physiology of velocity-sensitive (V) neurones in the fCOv. (Bi) Response of a velocity-sensitive unit (V+) to an elongation of the fCO. The relationship between the activity of this neurone and the stimulus velocity is given in Bii (large filled circles). The relaxed fCOv position corresponded to approximately 130 ° and the elongated position corresponded to approximately 90 °. (Bii) Dependence of the activity of nine different (V+) neurones on the velocity during elongation of the fCOv. Velocity values are given both as mm $s^{-1}$ and as degrees $s^{-1}$. Note the different slopes of the lines (see text for details). (C) Physiology of two acceleration-sensitive (A) neurones in the fCOv. (Ci,ii) Positive acceleration values above 1000 $\mu$m $s^{-2}$ elicited activity in an A+ neurone. (Ciii,iv) Negative acceleration values in excess of −1500 $\mu$m $s^{-2}$ elicited spikes in an A− unit of the fCOv. SN, sensory neurone; Pos, position trace of the fCO stimulus; Vel, velocity trace of the fCO stimulus; $a_s$, $a_e$, acceleration values at the start ($a_s$) or end ($a_e$) phase of a stimulus.

neurone showed peak activity near the 90 ° position of the fCOv (Pm; not shown; see Table 1). Most of the recorded position-sensitive neurones showed hysteresis, as described previously (Hofmann *et al.* 1985; Matheson, 1990; Zill and Jepson-Innes, 1988). This is clearly visible for the neurone shown in Fig. 2Ai,ii. The mean activity of this sensory neurone at any given fCOv position was higher when this position (e.g. +400 $\mu$m in Fig. 2Ai) was reached from a more relaxed position than it was when the position was reached from a more elongated one.

Twenty-nine sensory neurones responded only to the velocity of the movement of the fCOv (i.e. they responded during the ramp part of ramp-and-hold stimuli). 70 % were spontaneously active at frequencies of 0.1–20 Hz (see also Ramirez *et al.* 1993). They were excited by the velocity of a stimulus during elongation (V+, Fig. 2B), during relaxation of the fCOv (V−), or during movements in both directions (V±). Their mean activity during a ramp stimulus increased in all cases with increasing stimulus velocity. A quantitative analysis of the relationship between stimulus velocity and the firing frequency of the V− units revealed two different slopes ($P$<0.01, modified $t$-test according to Dixon and Massey, 1969; Fig. 2Bii): (i) a steep curve with an average slope of 237.8±73.4 spikes $s^{-1}$ $decade^{-1}$ (S.D., $N$=5; range 170–300 spikes $s^{-1}$ $decade^{-1}$) and (ii) a less steep curve with an average slope of 81.8±6.2 spikes $s^{-1}$ $decade^{-1}$ ($N$=4; range 74–90 spikes $s^{-1}$ $decade^{-1}$). Velocity thresholds were not measured for all recorded neurones but, when measured, ranged from below 125 $\mu$m $s^{-1}$ (12.5 degrees $s^{-1}$) to 2000 $\mu$m $s^{-1}$ (200 degrees $s^{-1}$). I did not investigate range fractionation of velocity-sensitive neurones (see Matheson, 1992).

Eighteen neurones responded solely to the acceleration component of a movement of the fCOv. During ramp-and-hold stimuli, accelerations occur only at the beginnings and ends of the ramps. Acceleration-

Table 1. *Summary of the number of neurones of each response type recorded in the fCOv of the stick insect*

| Classification | Response | Number of records | Comparative values |
|---|---|---|---|
| Position-sensitive | P+ | 14 | 11 |
| | P– | 5 | 3 |
| | Pm | 1 | 3 |
| Position- and velocity-sensitive | P+,V+ | 3 | 13 |
| | P+,V– | 1 | 1 |
| | P–,V+ | 0 | 2 |
| | P–,V– | 1 | 3 |
| | P+,V± | 1 | 0 |
| | P–,V± | 1 | 1 |
| Position- and acceleration-sensitive | P+,A± | 1 | 0 |
| Velocity-sensitive | V+ | 12 | 12 |
| | V– | 9 | 10 |
| | V± | 8 | 8 |
| Velocity- and acceleration-sensitive | V+,A± | 4 | 0 |
| | V+,A– | 0 | 2 |
| | V–,A+ | 1 | 0 |
| | V–,A– | 0 | 4 |
| | V–,A± | 2 | 0 |
| | V±,A± | 0 | 5 |
| Acceleration-sensitive | A+ | 1 | 0 |
| | A– | 1 | 12 |
| | A± | 16 | 16 |

For comparison, I present the numbers of each type recorded by Hofmann and Koch (1985).
P, position; V, velocity; A, acceleration (for details see text).

sensitive neurones responded to positive acceleration values (A+, Fig. 2C), to negative acceleration values (A−, Fig. 2C) or to both acceleration directions (A±; see Table 1). The acceleration thresholds of investigated neurones ranged from $0.006\,m\,s^{-2}$ to $8.5\,m\,s^{-2}$. Most of the recorded neurones responded to both acceleration directions (Table 1). In these neurones the thresholds were often lower for negative accelerations than for positive accelerations (see also Hofmann and Koch, 1985).

Fifteen sensory neurones in the fCOv responded to combinations of the stimulus variables position, velocity and acceleration. Five neurones responded to both position and velocity, one responded to position and acceleration and three responded to velocity and acceleration.

The locations of the somata of three velocity-sensitive neurones and four acceleration-sensitive neurones were determined by injecting Lucifer Yellow into their axons following physiological characterisation. The dye was injected using hyperpolarising current pulses (−10 nA, 600 ms, 1 Hz) for 25 min. A diffusion time of 60–90 min was

given after finishing the dye injection. The somata of all seven units were located in the fCOv (Fig. 1B,C). This excludes the possibility that a mechanical coupling between both fCO scoloparia, either at their common base or through the surrounding saline, led to stimulation of sensory neurones that were not located in the fCOv, but in the cut and isolated dorsal part of the fCO (fCOd).

This investigation has shown that the ventral scoloparium of the fCO, which acts as the transducer for the FT control loop, contains sensory neurones that respond to the position, velocity or acceleration of a movement or to various combinations of these movement variables. These results are in accordance with those of Hofmann *et al.* (1985) and Hofmann and Koch (1985), who stimulated the whole fCO (see Table 1). Most types of fCO sensory neurones were recorded in both studies. A marked difference from the results of Hofmann *et al.* (1985) only occurred in the number of recorded A− units (Table 1). Neither Hofmann and Koch (1985) nor I found any receptors responsive to position, velocity and acceleration in the fCOv. For the locust metathoracic femoral chordotonal organ, which is not separated into two morphological distinct scoloparia, Matheson (1990) described a set of sensory neurones that is sensitive to position, velocity and possibly to acceleration. However, in his study, acceleration sensitivity could not be proved because of methodological restrictions (Matheson, 1990; Matheson and Ditz, 1991).

My results show that sensory neurones within the fCOv measure the position, velocity and acceleration of the FT joint. The recently described functional specialisation of the fCO scoloparia (Kittmann and Schmitz, 1992) does not, therefore, affect our current understanding of the detection of these variables and their processing in the FT control loop. However the question remains as to what function the dorsal scoloparium of the fCO, which contains the majority of the sensory neurones, serves. Extracellular (Field and Pflüger, 1989) and intracellular recordings (A. Büschges, unpublished results) indicate that the dorsal part of the fCO might detect vibrations.

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