

THE EXOSKELETON AND INSECT PROPRIOCEPTION

III. ACTIVITY OF TRIBAL CAMPANIFORM SENSILLA DURING WALKING IN THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA*

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SUMMARY

1. In the cockroach tibia, the activities of campaniform sensilla that monitor cuticular strain have been recorded in free-walking animals.

2. In walking, sensillum firing is correlated with myographic activity of the flexor and extensor tibiae muscles.

3. The specific activity of a single campaniform sensillum depends upon the orientation of its cuticular cap.

4. In slow walking, proximal sensilla, whose ovoid cuticular caps are oriented perpendicular to the leg long axis, fire in bursts that are initiated just prior to the onset of extensor tibiae activity in the stance phase of locomotion. The firing frequency within bursts of proximal sensilla is generally inversely related to the frequency of the slow extensor tibiae motoneurone and ceases when motoneurone activity exceeds 200 Hz.

5. Distal campaniform sensilla, oriented parallel to the leg long axis, only fire when slow extensor tibiae activity exceeds 300 Hz. In slow walking, distal sensillum activity typically occurs as a short intense burst near the end of the stance phase of the step cycle, when slow extensor frequency is maximal. Distal sensillum firing is greatly increased when forward progression is impeded.

6. The patterns of afferent activity seen in slow walking indicate that the campaniform sensilla function in load compensation and limitation of muscle tensions. The proximal sensilla respond to initial loading of the leg and can reflexly excite the slow extensor motoneurone in compensation. The distal sensilla respond to cuticular strains that result from large extensor contractions and can reflexly inhibit the slow motoneurone.

7. In rapid walking, activities of both subgroups of campaniform sensilla shift in phase relative to slow extensor firing. Proximal sensilla activity occurs after the onset of slow extensor firing. Distal sensilla bursts follow the termination of slow extensor activity.

8. These phase shifts limit the reflex functions of the tibial campaniform sensilla in rapid walking. Shifts in phase of afferent activity may contribute to the need for central programming of locomotion.

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INTRODUCTION

In insects, the central generation of walking patterns has been strongly suggested by a number of studies (Hoyle, 1976; Fournier, 1976). Patterns of activity that are characteristic of walking may be elicited in insect leg motoneurons despite deafferentation (Hoy & Wilson, 1969; Pearson & Iles, 1970). Central interneurons that can drive leg motoneurons in these bursting patterns have also been identified (Burrows & Siegler, 1976). Imposed depolarization of some of these interneurons resets motoneuron bursting rhythm, implying that these interneurons may be primary pattern generators (Pearson & Fournier, 1975).

Much less is known about how leg sense organs affect centrally generated walking patterns (Mill, 1976). Several experimental observations suggest that, at slow to moderate walking speeds, locomotor patterns are modulated to compensate for variations in load. For example, in freely moving cockroaches, motoneurons to extensor muscles (muscles that support the body weight in upright walking) increase their firing rate when more load is applied, as when other legs are lifted from the ground (Pearson, 1972). The same motoneurons show only irregular activity in deafferented preparations (Pearson & Iles, 1970), suggesting that their activity is strongly influenced by sensory feedback but only weakly driven by pattern-generating interneurons (Pearson & Fournier, 1975). Amputations of legs of cockroaches produce immediate changes in position and use of the remaining legs in locomotion (Wilson, 1966; Delcomyn, 1971). These changes are related to the increased proportion of the body weight borne by each of the remaining legs (Hughes, 1957). These experiments imply that leg sense organs provide information about loading of the leg and can rapidly adjust locomotor patterns accordingly.

Other experiments suggest that sense organs may be less effective in modulating locomotor patterns in rapid walking. In rapid walking of many animals the timing of motoneuron bursts is altered with respect to the movements produced (Delcomyn, 1969; Burrows & Hoyle, 1973; Grillner, 1975; Hoyle, 1976). Delcomyn and Usherwood (1973) studied this problem in cockroaches by simultaneous electromyography and high-speed cinematography. In slow walking, leg motoneuron bursts nearly coincided with the movements they produce. In rapid walking, however, motoneuron bursts occurred far in advance of movement and often were terminated before the movement ended. Much of the energy imparted by leg muscles was then expended in dampening movement, a phenomenon widely observed in vertebrates (Basmajian, 1962; Engberg & Lundberg, 1969). These motoneuron phase shifts suggest that afferent activity that either signals movement or detects load occurs at different times, relative to motoneuron bursting, in slow and fast walking. Since motoneuron bursts are quite short in rapid locomotion (Pearson, 1972; Krauthamer & Fournier, 1978) the reflex effects of these sense organs might be so delayed as to substantially reduce their effectiveness (Hoyle, 1976).

The specific activities and effects of leg sense organs that can detect load have remained undetermined in free-walking insects. The present study investigates whether the tibial campaniform sensilla exhibit patterns of activity in walking animals and whether such activity could modulate locomotor patterns in walking, by recording the activity of tibial campaniform sensilla and the extensor and flexor tibiae muscle

in freely moving animals. While many studies have monitored myographic activity in walking animals (Hoyle, 1964; Pearson, 1972; Ayers & Davies, 1977) few experiments have attempted direct recording of afferent activity from leg nerves (Runion & Usherwood, 1966, 1968).

The preceding studies (Zill & Moran, 1981; Zill, Moran & Varela, 1981) that characterized the adequate stimuli and reflex connexions of these receptors concluded with the following specific hypotheses as to their function.

The proximal tibial campaniform sensilla, whose ovoid cuticular caps are oriented perpendicular to the long axis of the leg, detect leg loading in upright walking and reflexly excite extensor motoneurons in compensation.

The distal tibial campaniform sensilla, oriented parallel to the leg axis, respond to large resisted contractions of the extensor tibiae muscle and reflexly inhibit slow extensor motoneurons.

The results of the following experiments substantially support these hypotheses and also suggest that afferent feedback may be less effective in rapid locomotion.

MATERIALS AND METHODS

Preparation

Adult American cockroaches of both sexes were used in these free-walking experiments. Animals were first briefly anaesthetized with carbon dioxide. The wings were then removed and the animals were placed, dorsal surface down, on a plastic block coated with Sylgard resin. Movement was prevented by a small insect pin placed through the rostral pronotum and by a series of staples of different sizes placed over the legs.

The right metathoracic leg, from which recordings were taken, was placed upon a thin plexiglass plate and restrained by a large staple over the coxa and a smaller staple over the distal femur.

Placement of recording electrodes

Insulated 50 μm copper or silver wires were used as recording electrodes (Hoyle, 1964; Krauthamer & Fourtner, 1978). Six pieces of this wire, each 3 feet in length, were twisted together and coated with a very thin layer of butyl rubber (Union Supply Co., Denver, Colorado) to within one inch at each end. At one end, these wires were individually soldered to connecting leads for amplification and recording. A very small drop of sticky wax on the dorsal edge of the right metathoracic femur attached the other end of the recording wires, at the point where the butyl rubber coating ended.

Small pairs of holes were made with a sharp insect pin in the ventral femoral cuticle over the extensor and flexor tibiae muscles (Dresden & Nijenhuis, 1953) and over the dorsal nerve (n5r8, Nijenhuis & Dresden, 1956) (Fig. 1a). The recording wires were then splayed apart, cut back and individually placed into these holes. Only the tips of the wires were uninsulated.

Positioning of the electrodes to achieve good signal-to-noise ratios and minimal cross-talk was aided by amplification and display of recordings at the time of electrode placement. The myographic electrodes slightly penetrated (0.5 mm) the underlying

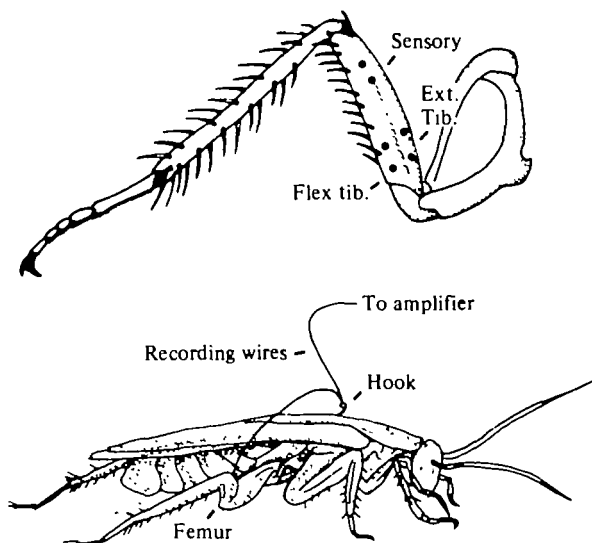


Fig. 1. Diagram of free-walking preparation. (a) Position of holes for myographic and neurographic recording. For myographic recording pairs of holes were made in the proximal femur near its dorsal (extensor tibiae) and ventral (flexor tibiae) edges. For neurographic recording a pair of holes was made in the distal femur near its midline. (b) Free-walking preparation. The recording leads were waxed to the dorsal edge of the femur and then tied to a small hook placed in the animal's back (mesonotum). The leads were then led from the animal for amplification, display and data storage.

muscles. Placement of the neurographic electrodes near the dorsal nerve was aided by visualization of a large central trachea that courses adjacent to nerve 5r8 (Fig. 2). This trachea was sharply delineated when transversely illuminated by a fibre-optic light source. The neurographic electrode wires were fed into the holes in the cuticle until they just reached the dorsal surface of the trachea. After positioning, all recording wires were fixed to the ventral surface of the femur with cyanoacrylate glue (Super Glue 3).

The animal was then re-anaesthetized and the restraining pin and staples were removed. The animal was rapidly turned over on its ventral surface and a small hook, made from a bent insect pin, was inserted into the dorsal mesonotum (Pearson, 1972) and fixed in place with sticky wax. The recording leads were tied to this hook by a small piece of thread (Fig. 1b). A copper wire soldered to the hook served to ground the animal.

The recording wires, hook, wax and glue added no more than 80 mg to the weight of the animal (mean weight 810 mg \pm S.E. 30).

Free walking

The animal was then placed in the walking chamber, a box constructed with plexi-glass walls and fine metal screening for its floor (Hoyle, 1964). The recording wires were tied to a rod that projected over the box and were left sufficiently long to permit free traversal of the entire walking area (24 in \times 30 in). A coating of vaseline on the inner surface of the walls discouraged the animal's occasional attempted departures.

Cockroaches generally showed considerable spontaneous locomotor activity at variety of walking speeds. Animals were judged to be walking normally by visual

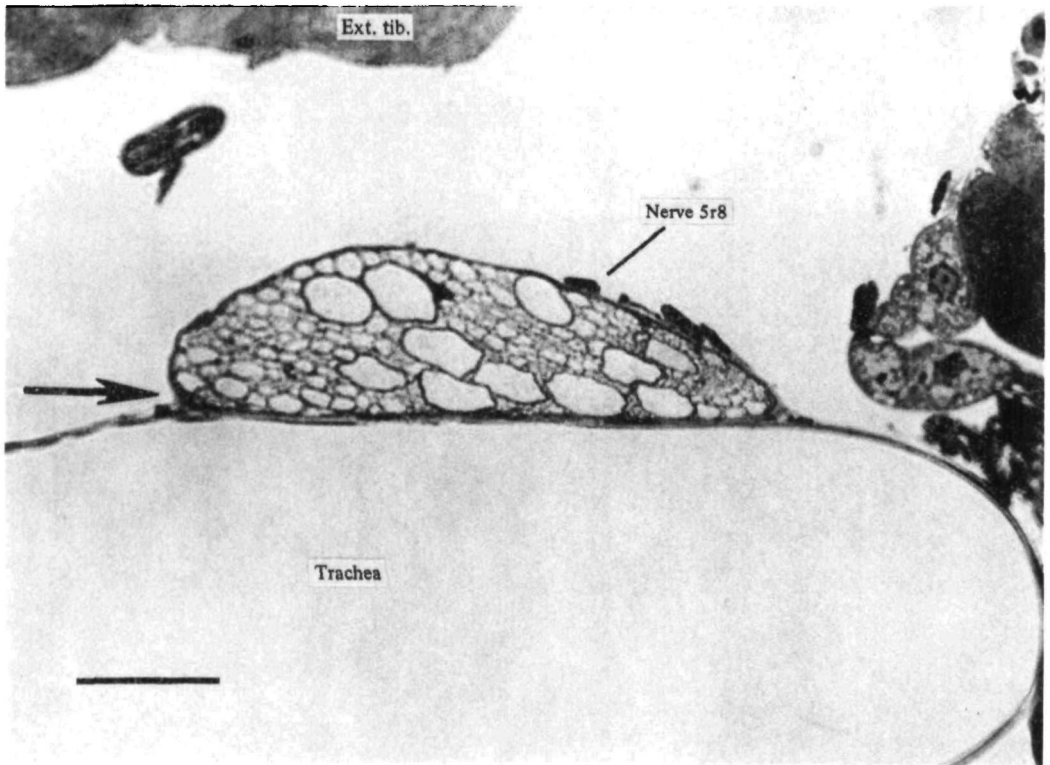


Fig. 2. Location of the dorsal nerve in the femur. Activity of some of the tibial campaniform sensilla was recorded from the dorsal nerve (n5r8) shown in transverse section in this photomicrograph. The nerve runs adjacent to a large central trachea. This trachea was used as a guide in positioning the recording electrodes (arrow). The large axons in n5r8 derive from some of the tibial campaniform sensilla and tibial tactile spines. Magnification: $780\times$. Calibration: $25\text{ }\mu\text{m}$.

inspection and by the recorded patterns of myographic activity (Krauthamer & Fournier, 1978). Data from animals that had inadvertently been damaged in the course of preparation were discarded. Results were obtained from 14 of 44 attempted preparations.

Sense organ identification and ablation

After an initial bout of free walking the animal was re-anaesthetized. Single campaniform sensilla were identified and ablated as previously described (Zill & Moran, 1981). The animal was then re-anaesthetized and returned to the walking cage, and afferent discharge in the dorsal nerve was re-examined.

Control experiments

Several control experiments were performed to ensure that activity of the large tibial and femoral spines (Chapman, 1965; Chapman & Pankhurst, 1967), that also send axons into *nr8* (Dresden & Nijenhuis, 1958), was not recorded in walking animals. In these experiments (4 animals) tibial and femoral spines that produced afferent discharge in nerve *nr8* were ablated. These ablations produced no detectable change in recordings from the nerve during walking. This finding affirms the purely exteroceptive nature of these receptors (Pringle, 1940; Dethier, 1963).

The possibility of damage to the dorsal nerve in the course of campaniform sensillum ablation was excluded in other animals in which the tactile spines were left intact. The dorsal nerve courses immediately below the tibial campaniform sensilla (Schnorbus, 1971). In initial preparation of these animals, several spines located distal to Group 6 were individually stimulated with a small wire and their activity was recorded. This activity was retested after campaniform sensillum ablation in both pinned-down and freely moving animals. In all of these preparations tactile spine discharge remained unaltered, thus indicating the integrity of the dorsal nerve. This control also ensured that the implanted electrodes remained close to the dorsal nerve during free walking.

Data storage and analysis

Myographic and neurographic data were recorded on magnetic tape and analysed as previously described (Zill & Moran, 1981). Activity below 100 Hz was filtered out on the sensory nerve channel to minimize movement artifacts.

RESULTS

Activity of the Extensor and Flexor Tibiae Motoneurons in Walking

The tibial extensor and flexor motoneurons were reciprocally active in walking at stepping frequencies over 3–5 Hz (Fig. 3*a(ii)*). While the exact relationship between motoneurone activity and leg movement was not analysed, at moderate speeds slow extensor tibiae activity could be heard clearly in the audio monitor when the animal was observed to place its leg down in support and push back in propulsion. This observation is in complete agreement with the findings of Delcomyn & Usherwood (1973) from combined cinematographic and myographic recordings of the extensor muscle of the trochanter, and suggests that the extensor muscles of both joints operate synergistically in walking.

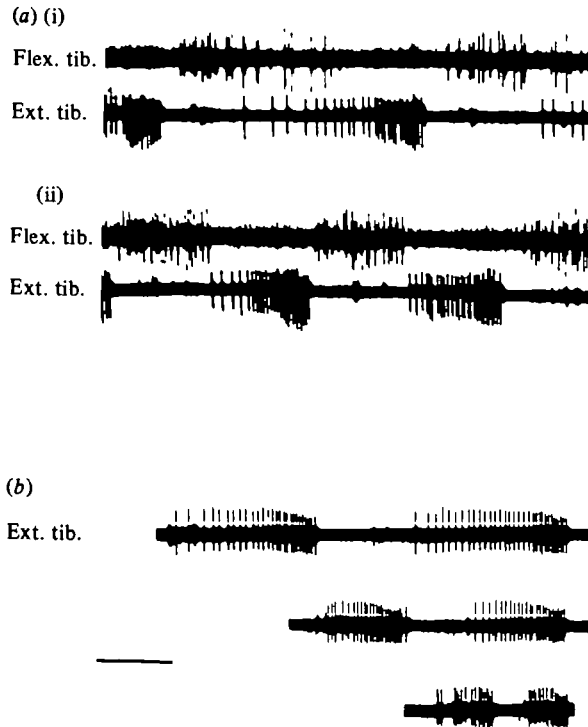


Fig. 3. Activity of the tibial motoneurons in walking. (a) Myograms were recorded from the flexor (upper trace) and extensor (lower trace) muscles at two different walking speeds. The extensor myograms show activity resulting from firing of the single slow extensor tibiae motoneurone. Single unit activity is not reliably identifiable in the flexor myogram. (i) Walking at 2 Hz shows some overlap of flexor and extensor activity. (ii) In walking at 3 Hz motoneurone activity is completely reciprocal. (b) Extensor bursting at different walking speeds. In slow walking (first and second trace) the extensor begins firing at a low rate and accelerates in a burst. In rapid walking (third trace) extensor activity becomes irregular within a burst. Calibration: (a) 135 ms; (b) 150 ms.

At walking speeds of less than 2 Hz strict reciprocity of antagonist activity did not occur in the tibial motoneurons (Fig. 3*a*(i)). Flexor activity apparently extended into the extensor burst and produced periods of co-contraction of variable duration. This co-activation occurs after placement of the leg on the walking surface (Krauthamer & Fournier, 1978) but its function remains unclear.

While single units were only occasionally identifiable on the flexor myogram, the slow extensor tibiae motoneurone was readily and unambiguously distinguishable in the extensor myogram. Slow extensor bursts were of long duration in slow-walking animals and uniformly decreased in duration in rapid locomotion (slope = 0.69 determined by the least squares method) (Fig. 4). This substantial decrease in extensor burst duration, correlated with a decrease in the duration of the stance phase, is characteristic of locomotion in many animals (Grillner, 1975; Hoyle, 1976).

The activity of the slow extensor motoneurone in a single burst formed a distinct and repeatable pattern (Fig. 3*b*). At slower walking speeds (less than 5 Hz) the slow extensor began firing at a low level (50–100 Hz), then accelerated and ended at a high firing frequency (300–400 Hz). The termination of a single burst was often abrupt but

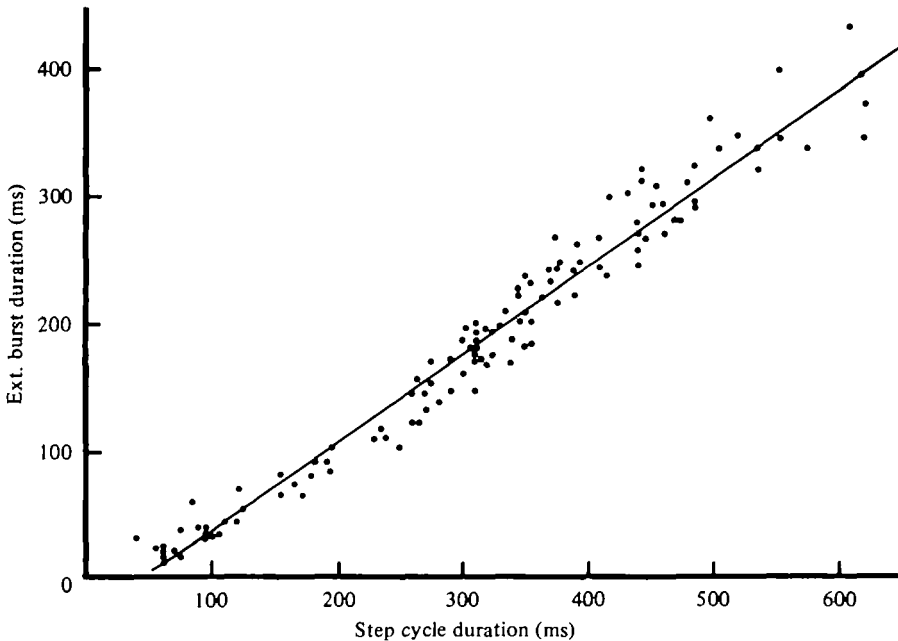


Fig. 4. Extensor burst duration at different walking speeds. Duration of the slow extensor burst is plotted for durations of the step cycle (longer durations are slower walking speeds). Extensor bursts decreased in duration as the animal moved more rapidly. These data closely agree with the findings of Pearson (1972) and Krauthamer & Fournier (1978) and were used as a criterion for normal walking.

in some cases, single spikes or pairs of spikes followed the burst at a much lower frequency. Uniform acceleration was absent at walking speeds of greater than 6–10 Hz, when extensor firing was initiated and maintained at a high frequency (over 200 Hz). This activity was irregular and often reached a maximum during the first half of the burst.

At walking speeds over 15 Hz fast extensor and flexor motoneurons were recruited. These motoneurons produced extensive cross-talk on all channels by conduction through the haemolymph, and neither sensory nor motoneurone activity could be unequivocally identified and analysed.

Activity of the tibial campaniform sensilla in walking at slow to moderate Rates

Recordings from electrodes placed close to the dorsal nerve in the femur (n5r8) exhibited patterned activity that was correlated with the discharges of the tibial muscles during locomotion.

Distal tibial campaniform sensilla

The largest afferent spikes in dorsal nerve recordings occurred in short groups near the end of slow extensor bursts (Fig. 5a). The following series of tests confirmed that the large distal tibial campaniform sensilla produced these spikes. After an initial free-walking period, the animal was re-anaesthetized and placed on the resin-coated

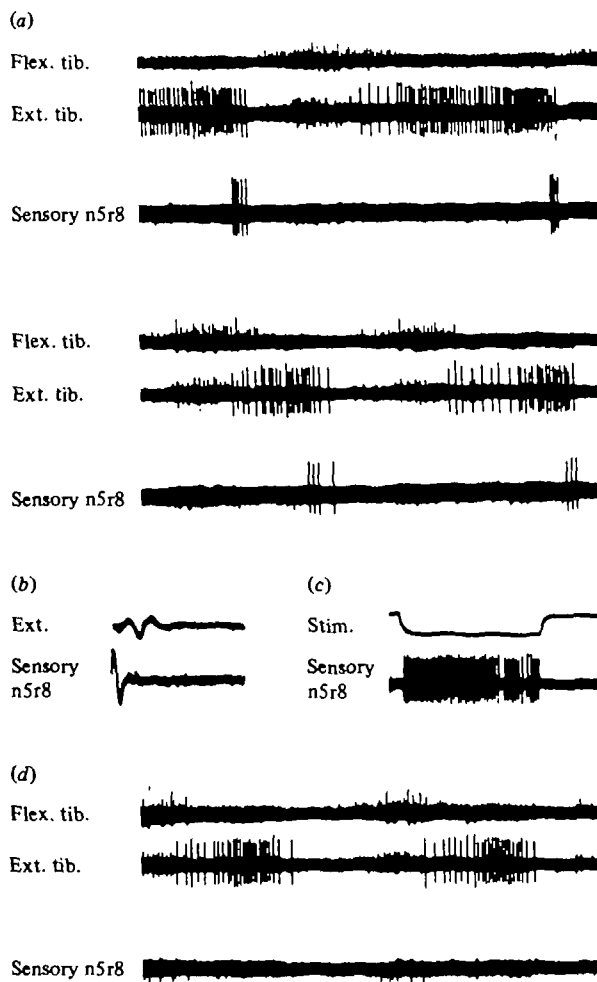


Fig. 5. Activity of the distal campaniform sensilla in walking. (a) Recordings of activity in a freely moving animal. Upper trace, flexor myogram; middle trace, extensor myogram; lower trace, recording of activity from the dorsal nerve (n5r8). Large-amplitude afferent bursts occur near the peak firing of the slow extensor at two different walking speeds. (b) Afferent cross-talk. Sensory spikes were used to trigger the oscilloscope sweep while the extensor myogram was displayed at high gain (ten traces are superimposed). The sensory spikes are followed 1:1 by small cross-talk spikes in the extensor channel. The delay indicates that this activity is afferent since it occurs in the more proximal myographic electrodes. (c) Stimulation of the caps of the Group 6 campaniform sensilla shows activity of only one large distal sensillum in the dorsal nerve. (d) Ablation of the cap of this sensillum eliminates activity at the end of extensor bursting. Calibration: (a, d) 200 ms; (b) 4.5 ms.; (c) 180 ms.

block. Cuticular caps of individual tibial campaniform sensilla were then mechanically stimulated using a fine tungsten wire affixed on a piezo-electric crystal. Stimulation of distal campaniform sensilla produced afferent spikes in the dorsal nerve recording, equivalent in amplitude to those seen in walking (Fig. 5c). The caps of these distal sensilla were then ablated with a sharpened heavy tungsten wire. The animal wa

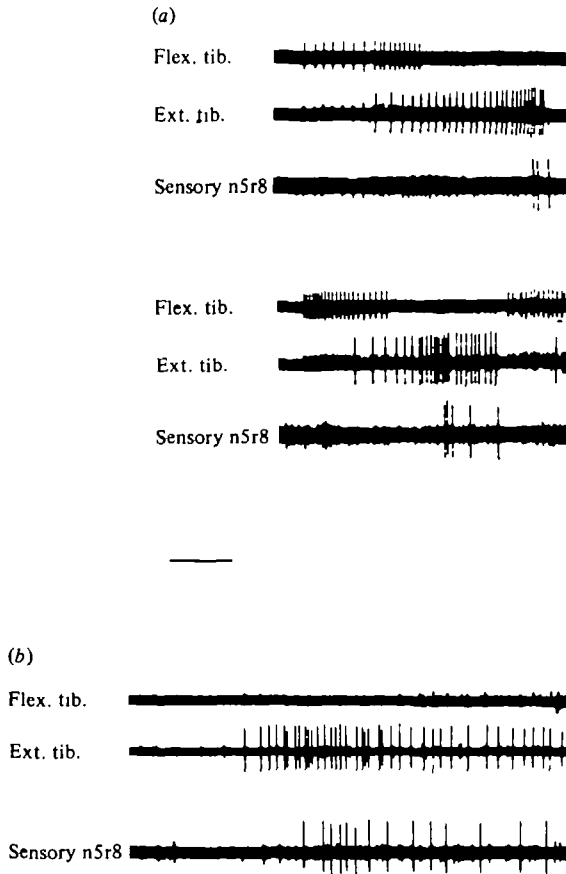


Fig. 6. Prolongation of distal sensillum firing. (a) A recording of a free-walking animal shows distal sensillum activity at the end of the extensor burst. In another step (lower set) extensor firing reaches a high level early in a burst. This firing evokes an early discharge from the distal sensillum. The extensor firing rapidly ceases and then is initiated at a lower frequency and is accompanied by further sensillum activity. (b) In this recording the animal had reached the end of the electrode leads and attempted to pull itself forward with an extensor muscle contraction. This attempted movement, that was completely resisted by the wires, produces a prolonged burst of distal sensillum activity. The initial intense firing of the sensillum is followed by a decrease in the rate of extensor activity. Calibration: (a) 100 ms; (b) 53 ms.

then reanaesthetized and returned to the walking chamber. Cap ablation of distal sensilla led to the absence of activity in the dorsal nerve at the end of an extensor burst (Fig. 5*d*).

These short bursts were the only afferent activity that was recorded when only distal sensillum axons travelled in the dorsal nerve (see Zill & Moran, 1981). In some of these cases, when recording was made from only a single distal sensillum, ablation of its cap eliminated all recorded afferent activity during walking. Also, spiking activity near the end of extensor bursts was unaffected by ablation of the tibial spines or the proximal sensilla (Fig. 7*d*). In some preparations, the large spikes of the distal sensilla produced low-level cross-talk in the extensor tibiae myogram. This cross-talk was conclusively afferent, since it appeared, with 1:1 congruence, after a delay in the

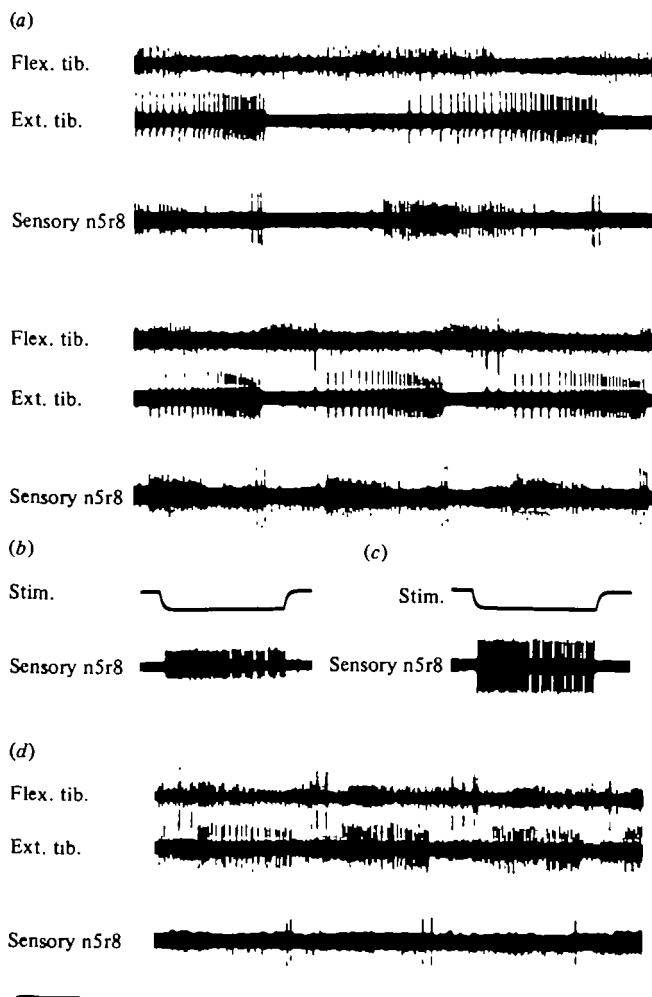


Fig. 7. Activity of the proximal sensilla in walking. (a) Recording from a freely moving preparation. Upper trace, flexor myogram; middle trace, extensor myogram; lower trace, recording from nerve 5r8. Small spiking activity occurs immediately preceding an extensor burst in slow walking and is nearly coincident with the onset of extensor bursting at more rapid speeds. This is approximately the time when the animal places its leg upon the walking surface (Krauthamer & Fournier, 1978). Large afferent spikes again occur at the end of the extensor burst. (b) The smaller spikes are identical to those produced by punctate stimulation of a single proximal campaniform sensillum. (c) The large spikes are equivalent to those produced by stimulation of a single distal sensillum. (d) Ablation of the proximal sensillum eliminates spiking activity early in the extensor burst. Larger spiking activity is unaffected. Calibration: (a, d) 120 ms. Stimulus duration in (b, c) 400 ms.

myographic electrodes (Fig. 5*b*). This delay permitted conduction velocity measurements (mean $4.1 \pm \text{s.d. } 0.8$ m/s; see Chapman & Pankhurst, 1967) that were found to be identical in walking activity and upon cap stimulation.

Distal sensillum firing was typically intense in a burst (100–400 Hz). The number of spikes in a burst varied according to the speed of walking. During slow walking (less than 5 Hz), bursts consisted of 3–5 action potentials and lasted up to 30 ms. In mor

rapid walking, only 1–2 spikes occurred; these persisted at the highest stepping frequencies recorded (13–15 Hz).

The phase and duration of the distal sensillum bursts depended upon the rate and acceleration of the slow extensor motoneurone activity. In slow walking, distal sensilla typically fired only when the slow extensor motoneurone reached a level of activity greater than 300 Hz. This level of activity generally occurred at the end of the extensor burst but occasionally the motoneurone accelerated 'prematurely' and distal sensillum firing occurred earlier in the extensor burst (Fig. 6*a*). Such early accelerations were often followed by a brief cessation of extensor activity without flexor firing, and subsequent extensor firing accompanied by further sensillum activity. This early sensillum firing apparently followed the tension developed by the extensor muscle.

Distal burst duration was also dependent upon resistance to forward walking. This dependence was demonstrated when the recording leads were left too short to permit walking to the edge of the cage. When the animal reached a point where it was held back by the wires it would attempt to pull forward by extensor muscle contractions. These extensor bursts were completely resisted by the wires and produced prolonged distal sensillum firing (Fig. 6*b*).

Proximal tibial campaniform sensilla. In most recordings ($n = 11$ of 14) from the dorsal nerve, afferent activity also occurred earlier in the extensor burst (Fig. 7*a*). These spikes were always smaller in height than those of the distal sensilla. Tests similar to those performed on the distal sensilla confirmed that this early afferent activity resulted from firing of the proximal tibial campaniform sensilla. Early afferent activity could be mimicked in spike height by proximal sensillum cap stimulation (Fig. 7*b*) and was eliminated by proximal sensillum cap ablation (Fig. 7*d*). It was also unaffected by ablation of the tibial spines and produced afferent cross-talk with a high conduction velocity (mean $3.5 \pm \text{s.d. } 0.4$ m/s) (Chapman & Pankhurst, 1967).

Bursts of proximal sensilla were always more prolonged than those of distal sensilla. In slow walking the proximal sensilla initiated firing at a high rate (300–400 Hz), most often immediately prior to extensor firing (Figs. 7*a*, 8). The proximal sensilla continued to fire in the early part of the extensor burst and occasionally showed slight accelerations. In general, however, their subsequent activity was inversely related to that of the slow extensor motoneurone. As the extensor accelerated in firing rate, the proximal sensillum discharge declined, and entirely ceased when extensor firing rose above 200 Hz. The proximal sensilla did not exhibit further activity as long as the slow extensor maintained this level of activity.

Activity of the tibial campaniform sensilla in rapid walking

Distal sensilla

In rapid walking (over 7–10 Hz) the firing of the distal campaniform sensilla shifted in phase relative to the bursting of the slow extensor motoneurone. While at walking rates below 5 Hz the distal sensilla regularly began firing before the end of the extensor burst (Fig. 9*a*), above 7–10 Hz sensillum activity was delayed until after the completion of extensor bursting. Distal sensillum activity then progressively overlapped the onset of flexor bursting (Figs. 9*b*, 10). In general, this change of phase exhibited a dependence upon the rate of slow extensor activity and was less following extensor bursts at high frequencies. Distal sensillum firing could not be identified

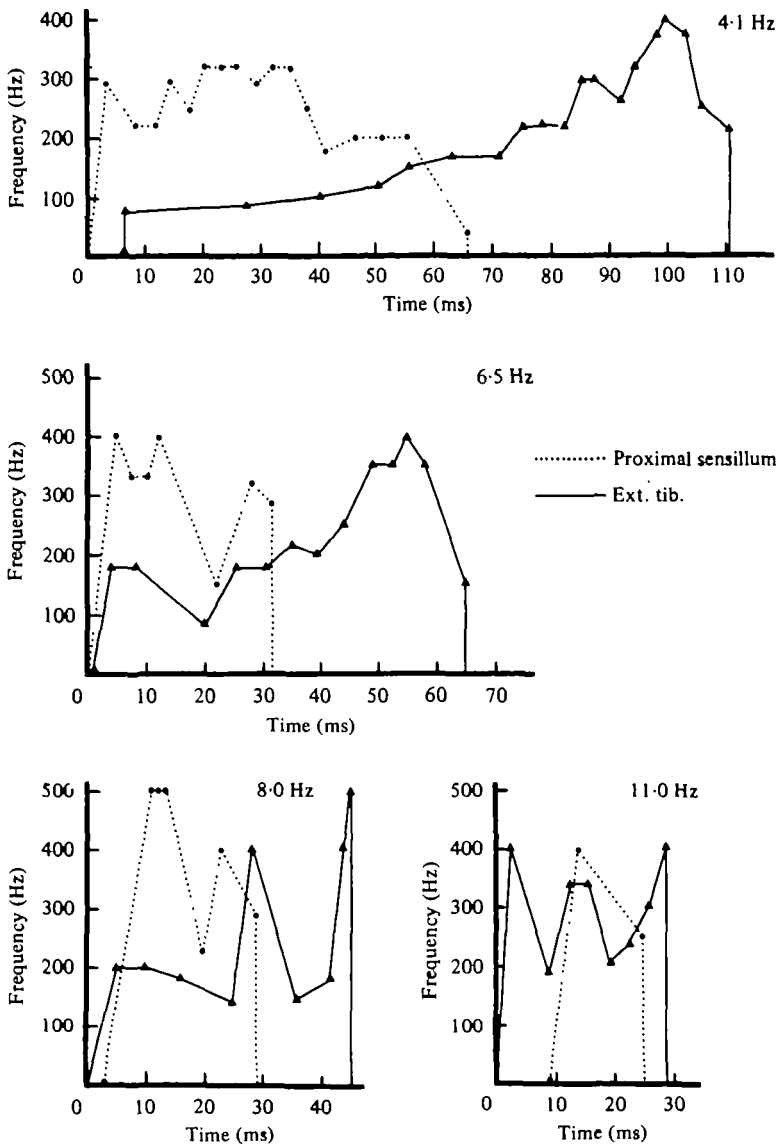


Fig. 8. Firing frequency of a proximal sensillum and the slow extensor tibiae motoneurone in steps at different walking speeds. Activity of a proximal sensillum and the slow extensor motoneurone were recorded simultaneously. This activity is plotted as the instantaneous firing frequency (calculated from measurements of inter-spike intervals) for four different steps taken at different walking speeds. At the slower walking speeds the proximal sensillum activity is initiated immediately prior to the firing of the slow extensor and shows reciprocity of activity with the extensor (4.1 and 6.5 Hz walking speeds). In faster walking (8.0 and 11.0 Hz) proximal sensillum activity follows the onset of extensor bursting. At the fastest walking speeds extensor bursting is irregular.

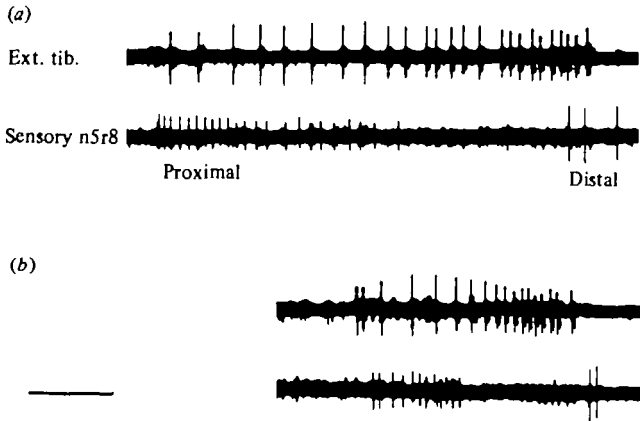


Fig. 9. Change in the phase of afferent activity at different walking speeds. Activity of the slow extensor (upper trace) and the tibial campaniform sensilla (lower trace) are shown for steps taken at slow (*a*) and fast (*b*) walking speeds. In slow walking the proximal sensillum firing is initiated immediately preceding the extensor burst. The distal sensillum fires before the end of extensor bursting. In rapid walking the proximal sensillum firing occurs after the onset of extensor activity. Distal sensillum activity occurs only after the extensor burst has terminated. Calibration: (*a*, *b*) 40 ms.

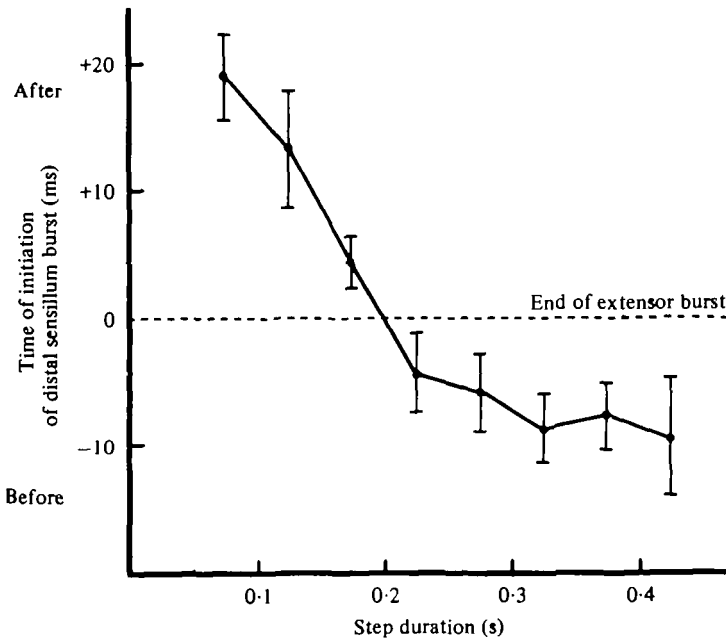


Fig. 10. Change in the phase of distal sensillum activity in rapid walking. The time of the initiation of distal sensillum activity relative to the end of the extensor burst is plotted on the ordinate (data from seven animals; error bars are s.e.). Negative values represent time before the end of extensor firing, positive values are time after the end of extensor activity. The duration of the step cycle is plotted on the abscissa for different walking speeds. At step durations greater than 0.2 s (walking at less than 5 Hz) the distal sensilla firing is initiated before the termination of extensor activity. At step durations less than 0.2 s (walking at greater than 5 Hz) distal sensillum lags behind the end of the extensor burst.

unequivocally in walking frequencies above 13–15 Hz, so the effects of fast motoneurone recruitment could not be examined. It should be noted, however, that the firing of the distal sensilla was never seen to shift beyond the following flexor burst and thus did not occur in the extensor bursts of the succeeding step.

Proximal sensilla

The activity of the proximal sensilla also shifted in phase relative to extensor bursting in rapid locomotion. In slow walking the sensillum firing immediately preceded the onset of extensor bursting (Fig. 9). Above 7 Hz, however, the extensor began firing before the proximal sensilla were active. At the maximum rates observed (13 Hz) the sensillum firing was initiated near the middle of the extensor burst and ceased before the extensor burst was complete.

DISCUSSION

The patterns of activity of individual tibial campaniform sensilla in freely moving animals depend upon sensillum cap orientation. This general finding is in complete agreement with the demonstrated orientation-dependent responsiveness and reflex connexions (Zill & Moran, 1981) of these receptors. Each of the activities of the subgroups of tibial sensilla, in posture and locomotion, can be correlated to their selectivity in response to strain.

Activity in walking at slow to moderate speeds

Proximal sensilla

In walking at slow to moderate speeds the proximal sensilla fire in a prolonged burst that is initiated just prior to the onset of extensor tibiae activity. The time of initiation of proximal sensillum firing occurs close to the point in time that the animal places its leg on the walking surface (Delcomyn & Usherwood, 1973). Subsequent afferent frequency is generally inversely related to the firing rate of the slow extensor tibiae motoneurone and ceases, in most cases, when motoneurone activity is maintained above 200 Hz.

Previous studies of the adequate stimuli of the proximal sensilla (Zill & Moran, 1981) have indicated that the proximal sensilla respond best to dorsal bending of the tibia and that their response is inhibited by contractions of the extensor tibiae muscle. These findings are consonant with the activity of the proximal sensilla in upright walking. A dorsal bending of the tibia should occur as the animal places its leg upon the walking surface and applies its weight. Dorsal bending produces proximal sensillum activity. As the animal supports its weight and provides propulsion by extensor muscle contractions, proximal sensillum activity would be inhibited. Precisely this pattern of activity is seen in upright walking. Combined high-speed cinematography and neurogram recording would be necessary to confirm the simultaneous occurrence of leg placement and proximal sensillum discharge.

A potential function of proximal sensilla firing in walking may be deduced from the reflex effects. The proximal sensilla excite extensor motoneurons. In walking at slow to moderate rates, the firing of the proximal sensilla could contribute to the initiation and acceleration of the extensor burst. The rate of extensor activity in a burst could

therefore be set to compensate for loading of the leg. The load compensatory function of the proximal sensilla is further examined and confirmed in a forthcoming paper that studies the effects of increased load and progressive sense organ ablations upon posture and walking patterns (S. N. Zill & D. T. Moran, in preparation).

The extensor contraction also reduces dorsal bending of the tibia and turns off the proximal sensilla. Thus this load compensatory system provides negative feedback, not the positive feedback postulated by Pringle (1961) and Pearson (1972).

Distal sensilla

During walking the distal tibial campaniform sensilla fire in discrete bursts that reflect activity in the extensor tibiae. Distal sensillum firing typically occurs at the end of a slow extensor burst when motoneurone activity is maximal but sensillum activity can be evoked by earlier motoneurone accelerations. The distal sensillum firing is more sustained when forward progression is impeded.

These patterns of activity are consistent with the finding (Zill & Moran, 1981) that distal sensillum activity can be evoked by resisted contractions of the extensor tibiae muscle. In those studies, however, distal sensillum firing could be evoked by levels of slow motoneurone activity of less than 100 Hz, while in free-walking animals distal sensillum firing is delayed until much higher frequencies of motoneurone activity are reached.

What is the cause of this discrepancy? A simple explanation could be that the tibia is first bent dorsally by the weight of the animal. Subsequently, higher levels of slow extensor firing have to be reached to overcome this loading before ventral bending and distal sensillum firing can occur.

Firing of the distal sensilla is regularly followed by a decrease in the rate of slow extensor motoneurone activity. This observation supports one of the functions assigned to these sensilla based upon their reflex effects: distal sensilla inhibit the slow extensor motoneurone and can prevent excessive muscle contractions. It should be noted that distal sensilla can also excite some flexor motoneurons. The discharges of these receptors at the end of extensor bursts could facilitate firing of some flexor motoneurons in the succeeding flexor bursts.

Thus the patterns of activity seen in the tibial campaniform sensilla in walking animals are consistent with the proposed hypotheses as to their function: load compensation and limitation of muscle tension. The alternate firing of the different subgroups of tibial sensilla can further be viewed as protecting the tibia and maximizing its use. Dorsal bending due to load can excite the proximal sensilla which in turn elicit contractions of the extensor muscle to straighten the tibia; ventral bending, that might be produced by overcontraction of the extensor, can be prevented by distal sensilla. Thus, the tibial group of campaniform sensilla, acting in concert, can ensure the structural integrity of the tibia in walking.

Activity in rapid walking

In walking at rates over 7–10 Hz, both subgroups of tibial campaniform sensilla shift in phase relative to the extensor burst.

Proximal sensilla

In slow walking the proximal sensilla begin firing prior to extensor bursting. In rapid walking sensillum activity is invariably initiated after the onset of extensor activity.

A reasonable cause for this late proximal sensilla firing can be found in the studies of Delcomyn (1969; Delcomyn & Usherwood, 1972) in which myographic and cinematographic data were recorded simultaneously. In rapid walking, Delcomyn found that motoneurone bursting shifted phase relative to the movement produced. According to his data on the trochanteral extensor muscle, at walking rates of 10 Hz the leg is not placed on the walking surface until the middle of an extensor burst. The earlier contractions of the extensor muscle were assumed to dampen or 'brake' the forward movement of the tibia. This shift in phase of motoneurone activity relative to leg placement in support corresponds closely to the altered time of proximal sensillum firing in rapid walking.

Distal sensilla

In rapid walking distal tibial campaniform sensillum activity also shifts in phase in the step cycle. Distal sensilla fire prior to the termination of extensor bursts in slow walking but reach spiking thresholds only after extensor bursting ends in rapid walking.

There are several possible causes for the shift in phase of distal sensillum activity. First, as previously reported (Zill & Moran, 1981), there is a substantial latency between the onset of slow extensor motoneurone activity and response of the distal campaniform sensilla even in preparations with movement completely resisted. Slow extensor firing at rates as high as 300–400 Hz could not apparently reduce this latency to less than 30 ms. In very rapid walking the slow extensor burst often did not exceed 25 ms. in its entire duration. Thus, in rapid walking, the apparent time necessary for tension development to elicit distal sensillum activity is greater than motoneurone burst duration, and a shift in phase is implicit. Second, as noted previously, in upright walking loading initially excites the proximal sensilla. This finding implies that the tibia is bent dorsally. Some of the initial force developed by the extensor muscle may be exerted to overcome this loading before ventral bending and distal sensillum firing can occur. Last, as noted for the proximal sensilla, the firing of motoneurons to leg muscles is advanced in phase in rapid locomotion (Delcomyn, 1969). Some of the energy of extensor muscle contractions could be expended in 'braking' the forward movement of the tibia before leg placement on the walking surface. This factor would also contribute to a delay in distal sensillum firing.

Possible functions of the tibial campaniform sensilla in rapid walking

The phase shifts in discharge of the tibial campaniform sensilla impose limitations on the potential ability of these receptors to modulate motoneurone activity in rapid walking. The late onset of proximal sensillum firing in rapid walking denies these receptors the ability to aid in the initiation of extensor bursting. Some modulation of motoneurone activity could occur during the short period of co-activity of the proximal sensilla and the slow extensor, but firing at the end of a sensillum burst often occurs

too late to modify extensor motoneurone activity effectively. Similarly, the activity of the distal sensilla that could aid in termination of the extensor burst in slow walking is evoked only when extensor activity in a step is complete in rapid walking. While distal sensillum activity could not terminate extensor bursting in rapid locomotion it could still contribute to activity of some flexor motoneurons in the lifting of the leg that follows.

Thus, while the tibial campaniform sensilla can make some contribution to the modulation of motoneurone activity in rapid walking this contribution is limited by the shift of phase relative to motoneurone bursting.

It is of interest to note that the shifts in phase of afferent feedback are accompanied by changes in the pattern of slow extensor activity within a burst (Fig. 3*b*). In slow walking, the extensor began firing at a low level and accelerated later in a burst. What is the advantage of this pattern of activity? In walking, the metathoracic leg is simultaneously retracted by muscles attached to the body wall while its joints are extended by intrinsic muscles (Hughes, 1952). High levels of activity late in an extensor burst, when the leg is partially retracted, would maximize the force exerted in forward propulsion. This uniform acceleration of extensor firing did not occur in rapid walking. The animal may thus sacrifice some efficiency in the use of muscle force in acquiring extreme quickness of movement. This potential loss of efficiency is accompanied by a decrease in the efficacy of reflex feedback from leg sense organs that are responsible for 'fine tuning' of motoneurone activity.

Peripheral feedback and central patterning of walking

The major conclusions of the present study are:

(a) Individual campaniform sensilla exhibit regular, repeatable discharges in locomotion that depend upon sensillum cap orientation and directional sensitivity.

(b) These patterns of activity could aid in load compensation and the limitation of muscle tension through reflex effects upon motoneurons during slow and moderate walking.

(c) These functions are probably limited in rapid locomotion.

In sum, the tibial campaniform sensilla apparently can serve to modulate and adapt walking patterns.

The findings of this study are compatible with the concept of locomotion as a centrally generated pattern that is adaptively elaborated by sensory feedback (Grillner, 1975; Hoyle, 1976; Pearson, 1972). Furthermore, the limited effectiveness of afferent feedback in rapid locomotion may itself contribute to the need for central programming. While the tibial campaniform sensilla can generate adaptive feedback at the walking rates that were studied, their effectiveness might be substantially reduced in more rapid walking. Cockroaches have been reported to walk at rates as rapid as 24 Hz (Pearson, 1972). At these rates, receptors that monitor joint angle and the campaniform sensilla, which monitor cuticular strain, may be unable to provide feedback with sufficient rapidity to modulate motoneurone activity effectively. Indeed, Hoyle (1976) has argued that central interneurone inhibition of afferent feedback is necessary in rapid walking to prevent disruptive reflex effects. While the need for such interneuronal inhibition has not been demonstrated in the present study, the shift in phase of afferent feedback may pose a formidable problem in the generation of rapid walking.

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REFERENCES

- AYERS, J. L. & DAVIS, W. J. (1977). Neuronal control of locomotion in the lobster, *Homarus americanus*. II. Types of walking leg reflexes. *J. comp. Physiol.* **115**, 29-46.
- BASMAJIAN, J. V. (1962). *Muscles Alive*. Baltimore, Maryland: Williams and Wilkins.
- BURROWS, M. & HOYLE, G. (1973). The mechanism of rapid running in the ghost crab, *Ocypode ceratophthalma*. *J. exp. Biol.* **58**, 327-349.
- BURROWS, M. & SIEGLER, M. V. S. (1976). Transmission without spikes between locust interneurons and motoneurons. *Nature Lond.* **262**, 222-225.
- CHAPMAN, K. M. (1965). Campaniform sensilla on the tactile spines of the legs of the cockroach. *J. exp. Biol.* **42**, 191-203.
- CHAPMAN, K. M. & PANKHURST, J. H. (1967). Conduction velocities and their temperature coefficients in sensory nerves of the cockroach leg. *J. exp. Biol.* **46**, 63-84.
- DELCOMYN, F. (1969). Reflexes and locomotion in the American cockroach. Ph.D. dissertation, University of Oregon.
- DELCOMYN, F. (1971). Effect of limb amputation on locomotion of the cockroach, *Periplaneta americana*. *J. exp. Biol.* **54**, 453-469.
- DELCOMYN, F. & USHERWOOD, P. N. R. (1973). Motor activity during walking in the cockroach, *Periplaneta americana*. *J. exp. Biol.* **59**, 629-642.
- DETHIER, V. G. (1963). *The Physiology of Insect Senses*. London: Methuen.
- DRESDEN, D. & NIJENHUIS, E. D. (1953). On the anatomy and mechanism of motion of the mesothoracic leg of *Periplaneta*. *Verh. K. Akad. Wet. C* **56**, 39-47.
- DRESDEN, D. & NIJENHUIS, E. D. (1958). Fibre analysis of the nerves of the second thoracic leg in *Periplaneta americana*. *Verh. K. Akad. Wet. C* **61**, 213-223.
- ENGBERG, I. & LUNDBERG, A. (1969). An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. *Acta Physiol. Scand.* **75**, 614-630.
- FOURNIER, C. R. (1976). Central nervous control of cockroach walking. In *Neural control of locomotion* (ed. R. Herman, S. Grillner, P. S. G. Stein and D. G. Stuart), pp. 401-418. New York: Plenum Press.
- GRILLNER, S. (1975). Locomotion in vertebrates: central mechanisms and reflex interaction. *Physiol. Rev.* **55**, 247-306.
- HOY, R. R. & WILSON, D. M. (1969). Rhythmic motor output in the leg motor neurons of the milkweed bug, *Oncopeltus*. *Fed. Proc.* **28**, 588.
- HOYLE, G. (1964). Exploration of neuronal mechanisms underlying behavior in insects. In *Neural Theory and Modelling* (ed. R. Reiss), pp. 346-376. Stanford University Press.
- HOYLE, G. (1976). Arthropod walking. In *Neural Control of Locomotion*. (ed. R. Herman, S. Grillner, P. S. G. Stein and D. G. Stuart), pp. 137-180. New York: Plenum Press.
- HUGHES, G. M. (1952). The coordination of insect movements. I. Walking movements of insects. *J. exp. Biol.* **29**, 267-284.
- HUGHES, G. M. (1957). The coordination of insect movements. II. The effect of limb amputation and the cutting of commissures in the cockroach (*Blatta orientalis*). *J. exp. Biol.* **34**, 306-333.
- KRAUTHAMER, V. & FOURNIER, C. R. (1978). Locomotory activity in the extensor and flexor tibiae of the cockroach, *Periplaneta americana*. *J. Insect Physiol.* **24**, 813-819.
- MILL, P. J. (1976). *Structure and Function of Proprioceptors in the Invertebrates*. London: Chapman and Hall.
- NIJENHUIS, E. D. & DRESDEN, D. (1956). On the topographical anatomy of the nervous system of the mesothoracic leg of the American cockroach. *Periplaneta americana*. *Proc. K. Ned. Akad. Wet. C* **58**, 121-130.
- PEARSON, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173-193.
- PEARSON, K. G. & FOURNIER, C. R. (1975). Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol.* **38**, 33-52.
- PEARSON, K. G. & ILES, J. F. (1970). Discharge patterns of coxal levator and depressor motoneurons in the cockroach, *Periplaneta americana*. *J. exp. Biol.* **52**, 139-165.
- PRINGLE, J. W. S. (1940). Reflex mechanism of the insect leg. *J. exp. Biol.* **17**, 8-17.
- PRINGLE, J. W. S. (1961). Proprioception in arthropods. In *The Cell and the Organism* (ed. J. A. Ramsay and V. B. Wigglesworth), pp. 256-282. London: Cambridge University Press.
- RUNION, H. L. & USHERWOOD, P. N. R. (1966). A new approach to neuromuscular analysis in the intact free-walking insect preparation. *J. Insect Physiol.* **12**, 1255-1263.

- SCHNORBUS, H. (1971). Die subgenualen sinnesorgane von *Periplaneta americana*: Histologie und vibrations-schwellen. *Z. Vergl. Physiologie* **71**, 14-18.
- WILSON, D. M. (1966). Insect walking. *Ann. Rev. Ent.* **11**, 103-122.
- ZILL, S. N. & MORAN, D. T. (1981). The exoskeleton and insect proprioception. I. Responses of tibial campaniform sensilla to external and muscle-generated forces in the American cockroach, *Periplaneta americana*. *J. exp. Biol.* **91**, 1-24.
- ZILL, S. N., MORAN, D. T. & VARELA, F. G. (1981). The exoskeleton and insect proprioception. II. Reflex effects of tibial campaniform sensilla in the American cockroach, *Periplaneta americans*. *J. exp. Biol.* **94**, 43-55.