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Detection of Forces and Body Load in Standing and Walking in the American Cockroach

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DETECTION OF FORCES AND BODY LOAD IN STANDING AND WALKING IN THE AMERICAN COCKROACH

DISSERTATION

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By
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INTRODUCTION

This thesis has two main goals. The first is to gain an understanding of how body weight is detected by sense organs of the legs. The second goal is to determine how this information influences muscle activities in standing and walking. The work will be presented as three separate chapters. Experiments will first be described which study how sense organs detect body load during standing. In chapter two, studies will examine how these sense organs respond when the effect of the load of the body is minimized during walking. Finally, the third chapter will describe experiments that utilize ablations to study how load detecting sense organs can affect motor activities during walking.

These studies have been performed on an insect, the American cockroach. Invertebrates are advantageous for experiments in sensory neurobiology as it is possible to record from the same group of sense organs in different animals (Bässler and Büschges 1998; Duysens *et al.* 2000; Libersat *et al.* 1987b; Zill 1990).These recordings allow for detailed observations to be made about sensory influences on behavior. Similar experiments would be difficult to perform in vertebrates (Prochazka 1996).

This thesis will focus primarily on sense organs that detect forces in legs. These types of sensory receptors can respond to changes in the effect of body weight upon the limbs (Delcomyn 1991; Duysens *et al.* 2000). In the literature on posture and locomotion, the term load is often used rather broadly and non-specifically. The term load can refer to body weight (Corna *et al.* 1999), forces resisting forward propulsion, (Dean 1991; Pearson 1972) and experimentally induced loads (ex. weights on ankles)

(Misiaszek *et al.* 2000; Yang *et al.* 1998). This thesis will specifically refer to changes in the effect of body load as delineated below. Body weight is the result of the action of gravity on the mass of the animal. In many animals, the weight of the legs is small in comparison to the parts of the body that are supported by the limbs (thorax, abdomen, and head) (Full 1997). In an animal that is standing or walking, these parts of the body produce substantial forces in the legs. Literature on human kinesiology refers to this as HAT load (weight of the Head, Arms and Trunk) (Norkin and Levangie 1992; Puniello *et al.* 2000). The effect of body load on the legs is relatively constant when standing still (Horak and Macpherson 1996). However, if an animal carries its offspring or food, or when a human lifts a package, there will be a net increase in the forces that develop in the legs as a result of the change in load. Furthermore, the effects of body load upon individual legs varies during walking (Chang *et al.* 2000; Full and Tu 1990; Full and Tu 1991). When an animal walks, it lifts some of its legs during the swing phase. This increases the percentage of the body weight and inertia that must be supported by the remaining legs (Pratt 1995). These variations in weight will also be described as changes in the effect of body load. In this thesis, a variety of techniques will be used to vary the effects of body load upon the legs.

In many animals, compensation for changes in load requires contractions of skeletal muscles of the legs and body (Schmitz 1993). The nervous system must adjust these contractions to the magnitudes and rates of change of load. Specialized sense organs that detect body load in the limbs have been described in all legged animals that have been studied . Studies in both vertebrate and invertebrate animals have examined how information from these sense organs affects muscle activities during standing and walking.

The first part of the following literature review will present a general overview of motor responses that compensate for changes in body load. The second section will describe sense organs that are capable of detecting load. The final sections of this introduction will review studies that have examined how information from load receptors can influence muscle activities during standing and locomotion.

Motor responses to changes in leg loading

Vertebrates and invertebrates are capable of carrying loads with little difficulty. For example, humans can carry heavy weights balanced on their head with only slight increases in energy expenditure (Kram 1991; Maloiy *et al.* 1986). Rhinoceros beetles can also walk quite normally while carrying loads of up to 30 times their own body weight (Kram 1996). Experiments will be described that show how animals respond to changes in loading by making accurate and efficient motor adjustments.

Responses to perturbation during standing

A number of studies have examined motor responses in humans when they are perturbed while standing . Individuals often respond by adjusting their posture to keep from falling. Most of these studies have used lateral perturbations of the substrate (Nashner *et al.* 1979). The effects of changing body load have only been examined indirectly in tests of responses to lateral displacements. Dietz studied how changes in leg loading can affect these types of motor responses by submersing humans in water to decrease the body load on the legs (Dietz *et al.* 1989). Motor activities that resulted from decreasing the load in this fashion were reduced in comparison to those seen in normally loaded subjects. These results suggest that in vertebrates, responses to perturbations of posture are influenced by the load on a leg.

Experiments using invertebrates have shown similar results. Changing the body height of a stick insect by pushing down on its back can affect the net force the legs produce on the ground. This force is produced by contractions of leg muscles and is changed as the height of the insect is raised or lowered (Cruse *et al.* 1993; Kemmerling and Varjú 1981). The load of the body had minimal effects on this force as the animal was supported above the substrate. If the animals in these experiments had their leg muscles pharmacologically relaxed, forces on the ground were not developed in response to imposed changes in height (Kemmerling and Varjú 1981). This indicates that the forces were developed specifically by contraction of muscles in the legs and that motor output is directly affected by imposed changes in body loading.

Responses to perturbation during walking

Another question that has been studied for many years is, how does the nervous system compensate for changes in load during locomotion? Walking consists of an alternation of movements of contralateral legs (Grillner 1975). The pattern consists of sequential stance (foot on ground) and swing (foot in air) movements. During walking, load may influence not only the level or magnitude of activity in muscles, but it can also determine changes in timing and transitions between the swing and stance phases.

A number of studies have demonstrated that modulations of motor activity occur when load is experimentally increased on legs in the stance phase.   For example, Stephens and Yang showed that increasing the body weight of adult humans (by wearing a belt containing diving weights) increased the amplitude of antigravity muscles during walking by as much as 134% (Stephens and Yang 1999). Similar increases in motor amplitude were seen during walking by pulling down on a rope attached to the body (Misiaszek *et al.* 2000). Uphill walking can produce similar increases in muscle activity as simply increasing the load on the leg. During uphill running in turkeys, muscle activities showed increases in burst amplitude (Roberts *et al.* 1997). Similar increases in motor activity were also found in cats when they walked on an incline (Carlson *et al.* 1998). The amplitude of muscle contractions can also decrease if loads are suddenly reduced. For example, it was shown that muscle activities decreased when animals stepped in a hole during free walking (Gorassini *et al.* 1994).

Motor responses to changes in load are also modulated with respect to timing (Yang *et al.* 1998). It has been shown (above) that adults responded to load during stance by substantially increasing the level of extensor motor activity. The results differed when similar studies were performed in infants. When infants (3 - 10 months) walked with increased weight on their legs, they adopted a different strategy of compensating for load than adults (Yang *et al.* 1998). There was an increase in both duration of the stance phase and the length of the entire step (stance to stance) cycle, but no comparable increase in extensor motor amplitude. Independent control of motor responses (amplitude and timing) may provide animals with greater flexibility to compensate for changes in load.

Invertebrates can display similar motor responses to changes in load (Duysens *et al.* 2000). Because many invertebrates are multi-legged animals (six, eight or ten legs), the change in load that occurs upon lifting of a single limb is often less because of the distribution of body load among the legs. A number of studies have demonstrated that invertebrates can adapt to load by changing motor activity and by shifting timing of gait. For example, when cockroaches dragged or carried weights while walking on a flat surface, activities in an extensor muscle showed enhanced firing frequency as well as changes in timing of motor bursts (Pearson 1972). Crabs that walked with weights attached to their body (to increase the load on the legs) adopted a strategy which used a lower overall height for walking in addition to lengthening the duration of the stance phase (Libersat *et al.* 1987a; Libersat *et al.* 1987b). Stick insects can also adjust motoneuron firing frequencies as well as timing of muscle bursts when subjected to changes in load (Cruse *et al.* 1993; Dean 1991).

Walking up a wall or inverted on a ceiling also has the effect of changing the loading on legs. Motor bursting has been shown to be adjusted with respect to timing when animals walk upside-down (Larsen *et al.* 1995). Thus, both vertebrates and invertebrates can adopt similar strategies of modulating the magnitude (frequency or amplitude) of muscle activities and/or adjusting timing of motor bursts to compensate for changes in load.

Sense organs that detect body load

Animals must be able to accurately detect the load of the body to produce appropriate behavioral and motor responses necessary for support. A simple method to determine the load of the body would be to measure forces produced upon the ground beneath the legs. This could be accomplished if animals had receptors in their feet that monitored forces as when weight is determined by a bathroom scale. However, animals do not possess sense organs that function in the same manner as a scale . Instead, they measure other variables that can be influenced by the load of the body such as muscle length and tension. These measurements may be advantageous because the forces on the ground are affected by the position of the legs and the posture of the animal (Full *et al.* 1991). These forces also depend on tensions developed by leg muscles. Sense organs that measure limb position (muscle length) and muscle tension could be used to help determine the effectiveness of compensation for body load. In addition, sense organs sensitive to pressure at the sole of the foot can also indicate that a change in load has occurred. Although the morphology and the physiology of the sense organs may be markedly different, both vertebrates and invertebrates possess receptors that detect muscle force, leg joint angles and substrate contact.

In vertebrates, Golgi tendon organs (GTO) respond to tension that results from muscle contractions (Duysens *et al.* 2000; Jami 1992). These receptors are located in series with muscle fibers in the area of transition between muscles and tendons. Tendon organs are innervated by Ib afferents which branch into many free nerve endings. When a muscle contracts, the collagen fibers which comprise the tendon, will straighten and compress the free nerve endings and cause the tendon organs to fire (Jami *et al.* 1985). Thus, tendon organs are not specifically sensitive to changes in body load, but can indicate variations in loading that are countered by muscle contractions.

Muscle spindles may also play a role in the detection of body load by determining changes in leg position . Muscle spindles are located within the muscle in parallel to other muscle fibers. They are small, encapsulated sensory receptors that are associated with specialized muscle cells. Muscle spindles have both sensory (Ia) as well as motor (gamma) innervation to their own (intrafusal) muscle fibers. Sensory information from muscle spindles can inform the nervous system of the length of a muscle. Gamma motor neurons may adjust the sensitivity of the muscle spindle by adapting the length of the spindle to the change in the (extrafusal) contractile muscle. Information from muscle spindles is used by the nervous system to indicate joint angles and the position of leg segments. Muscle spindles can help produce the correct motor pattern in response to perturbations by indicating changes in muscle length. This is important as muscles have different mechanical advantages at different lengths.

Sense organs located in the feet and ankles can also indicate loading of the body weight on the leg. These receptors are sensitive to changes in pressure and stretch of the skin (Duysens *et al.* 2000). Pressure sensitive sense organs may be important in signaling foot contact with the substrate. This type of information could play a role in initiating swing to stance transitions during locomotion (Gorassini *et al.* 1994).

Invertebrates utilize similar sensory modalities (force, muscle length) as vertebrates for detecting changes in body load (; Zill 1993). One way invertebrates sense changes in body load is by monitoring strains or forces developed in the exoskeleton (Klärner D. and Barnes W.J.P. 1986). There are a number of specialized receptors used to detect these forces (Klärner D. and Barnes W.J.P. 1986; Pringle J.W.S. 1961). One such type of sense organ is the campaniform sensillum.

Campaniform sensilla are located within the cuticle of the animal and are often found close to joints (Klärner D. and Barnes W.J.P. 1986; Pringle J.W.S. 1938). These sense organs respond to strains developed in the exoskeleton which result from both the load of the body as well as muscle tensions. A campaniform sensillum consists of a single bipolar neuron whose cell body is located in the epidermis (Moran *et al.* 1971).

The dendrite of the sensillum inserts onto an ovoid cuticular cap at the surface of the exoskeleton. Forces in the leg produce distortions of the cap which evoke discharges in the neuron. The sensilla also show directional responses and are most sensitive to forces that are perpendicular to the long axis of the ovoid cap (Zill and Moran 1981).

The tibial campaniform sensilla of the cockroach have been studied in great detail and many of their response properties to forces have been well documented (Ridgel *et al.* 1999; Ridgel *et al.* 2000). There are two subgroups of tibial sensilla, proximal and distal, which are named for their location on the leg. The two subgroups have perpendicular orientations of their caps. The responses of the proximal and distal sensilla reflect the cap orientations. The proximal sensilla respond to imposed bending forces that mimic loading of the leg during stance (Ridgel *et al.* 1999). Responses indicate not only the rate of change, but also the magnitude of force. Distal receptors can respond to decreasing levels of forces on the legs similar to those that may occur at the end of the stance phase (Ridgel *et al.* 1999).

Mechanoreceptors in other invertebrates have response properties similar to campaniform sensilla (Libersat *et al.* 1987b). Receptors in the dactyl (terminal limb segment) of the crab are sensitive to changes in strains developed in the exoskeleton. These receptors can also indicate the load of the body on a limb during standing and walking (Libersat *et al.* 1987a).

Invertebrates also utilize changes in leg position to aid in the determination of body load . An example of a sense organ that can detect joint angles is the chordotonal organ of arthropods (Zill 1985a; Zill 1985b). A chordotonal organ consists of a group of sensory neurons that are located in one leg segment and mechanically linked to an adjacent segment of the leg. The sensory neurons are stretched when the joint angle changes. Signals from the organ as a whole encode the position and velocity of joint movement. There are no sense organs comparable to muscle spindles in legs of invertebrates, but chordotonal organs can fulfill similar functions in posture and locomotion. Chordotonal organs may help to maintain posture by adjusting muscle activities through resistance reflexes that occur in response to changes in joint angles (see below) (Bässler and Büschges 1998; Zill 1985b).

When an animal walks, it must know if a leg has made contact with the substrate and is able to support the body during the stance phase, to allow other legs to lift into swing (Grillner 1975). In multi-legged animals, this is very important for rear legs that are not directly in the line of vision. Invertebrates possess cuticular hair cells that cover most of the body and appendages. These hair cells indicate that a leg has made contact with the surface during walking (Laurent and Hustert 1988).

Sensory modulation of muscle activity

How do sense organs contribute to motor activities during standing?

There are a number of sensory cues available to the nervous system to ensure stable support of the body (Horak and Macpherson 1996). Information from muscle force receptors (Golgi tendon organs) can modify contractions of leg muscles through reflexes. Reflex effects of Golgi tendon organs (clasp-knife reflexes) were originally thought to perform a protective function by inhibiting muscle contractions at very high tensions, which could tear the muscle from its tendon (Houk 1979). For example, when a weight lifter raises a heavy weight, muscles increase their activity to compensate for increased load. A reflex was thought to occur which inhibits further motor activity if the tension in the muscle became too high. This reflex would function as negative feedback from the tendon original to the motoneuron. More recently, it has been shown that Golgi tendon organs may function through other pathways that could aid in load compensation.

There is some indirect evidence that activities from tendon organs can enhance motor responses to changes in body load. Pratt showed that electrical stimulation of leg extensor muscles in freely standing cats could produce short latency excitatory responses in other leg muscles (Pratt 1995). This excitation was at a latency (delay) consistent with the idea that it was due to Golgi tendon organs. It was hypothesized that this motor response would be similar to adjustments normally made in response to increases in body load. To test this hypothesis, motor recordings were obtained from a number of muscles in cat legs during times of sudden changes in loading (Pratt 1995). Animals stood above a platform that allowed for the support under each foot to be lowered independently. When the platform was dropped from below a leg, the animal responded by increasing muscle activity in adjacent legs. Motor responses elicited from leg muscles in these experiments showed very similar patterns of activity to the responses seen in the electrical stimulation study. These results suggest that Golgi tendon organs may contribute to responses to increased body load.

Receptors that monitor muscle lengths and associated changes in joint angles can also contribute to compensation for variations in body load (Nashner 1976; Nashner *et al.* 1979). Muscle spindles elicit stretch reflexes in motoneurons to leg muscles. For example, when a leg is pushed towards extension, flexor muscles will be stretched. This will activate flexor muscle spindles that will, in turn, excite flexor motor neurons through a monosynaptic reflex arc. These reflexes could substantially contribute to adjusting motor responses to changes in leg loading.

Prochazka has recorded activities of muscle spindles in freely moving cats and shown that they can be activated when loads are suddenly changed. In one experiment, recordings were obtained from muscle spindles while a cat attempted to stand (rise up) from a crouched position (Prochazka *et al.* 1977). During unperturbed rising, the cat showed a level of firing in spindle afferents that indicated the change in muscle length of leg extensors. When a light thrust was applied to the back of the animal during rising, sensory recordings indicated an increase in the firing frequency of the muscle spindles. This example clearly indicates that changes in body loading can be indicated by spindle activity.

Other studies that have directly recorded activities of leg sense organs in freely moving animals have shown that both muscle receptors and Golgi tendon organs can be activated in load compensatory reactions. Humans display similar motor responses to cats when subjected to increases in body load (Aniss *et al.* 1990). In these experiments, humans supported themselves above a platform by holding a bar with their arms. Subjects could release the bar so the load of the body was lowered onto the legs. This often produced increases in extensor myogram activity. In addition, activities of individual Golgi tendon organs and muscle spindles were recorded from peripheral nerves. Both types of receptors fired during the time of increased leg loading.

Information from sense organs in the skin of the foot and ankle may also contribute to responses to changes in body load (Duysens *et al.* 2000). A number of experiments have tried to determine the role of cutaneous afferent input on muscle activity during standing. It was suggested that cutaneous information from the foot could have the effect of reinforcing supportive muscle activity during standing (Duysens *et al.* 2000), The pad and the plantar surface of the foot of a cat was electrically stimulated to test this hypothesis (Duysens and Pearson 1976). This stimulus resulted in increases in activities of extensor muscles in the leg. This supports the hypothesis that cutaneous input from the foot can influence motor activity in response to a change in body load. However, cutaneous afferent information may be more important in determining foot contact at the initiation of the stance phase of walking (see below).

The sense organs of invertebrates that detect changes in muscle force and in joint angles could readily detect variations in loading while they stand . These sense organs can also elicit reflexes that could contribute to the maintenance of posture while standing. For example, the proximal group of tibial campaniform sensilla have been shown to excite extensor motor neurons and inhibit flexor motoneurons. The distal tibial sensilla have opposite effects (Zill and Moran 1981). Sensory discharges of tibial sensilla can encode factors such as the rate of change in force on the leg which could inform the animal of how quickly the body load is changing (Ridgel *et al.* 2000). In the experiments described in this thesis, the hypothesis that sensilla encode the effect of body load on the legs will be directly tested by changing loads in freely standing animals.

Changes in joint angles in invertebrates can initiate reflexes that could serve to counter changes in load. Chordotonal organs that monitor joint angles can elicit resistance reflexes that oppose joint movement (Zill 1985a). For example, when body load is increased, leg joints may become more flexed. This joint flexion will be detected by chordotonal organs that will activate extensor muscles to maintain leg position (Spirito *et al.* 1972). In studies of resistance reflexes, the motor response was dependent on the magnitude and direction of movement (Zill *et al.* 1985). Thus, chordotonal organs can function like vertebrate muscle spindles in countering loads by monitoring changes in leg position.

These types of reactions may also be involved in regulation of body height. When an insect stands or walks on an uneven surface it needs to maintain a low center of gravity while keeping the body stable, even with legs at different heights and angles . Animals may regulate their height by sensing changes in joint angles. When stick insects were pressed down toward the substrate they responded to imposed changes in height by producing muscle forces which opposed vertical movements (Cruse *et al.* 1989). It is not known if receptors monitoring leg forces can contribute to the responses of stick insects. In this thesis, forces will be applied to freely standing cockroaches that produce changes in body height and result in vigorous discharges of campaniform sensilla that encode forces in the legs.

These examples illustrate how sense organs can influence the magnitude of a motor response during standing. To understand how sense organs can affect the timing of motor bursts in response to changes in load it is necessary to study these effects during locomotion.

Role of force/load sensors in walking

A number of sense organs can inform the nervous system of ongoing changes in body load during locomotion (Duysens *et al.* 2000). There are two types of motor adjustments typically made in response to changes in load during walking (Stephens and Yang 1999). The first is produced by changing the magnitude of the motor response, which can occur through alterations in motoneuron firing frequency or recruitment of additional motor units. Secondly, timing of motoneuron bursting may be advanced or delayed to compensate for changes in leg loading.

In vertebrates, Golgi tendon organs can contribute to modifications in magnitude of motor bursts during walking. Recordings from free walking cats show that tendon organ 1b afferents are active during the stance phase (Prochazka *et al.* 1977). In spinal cats, electrical stimulation of Ib afferents increases the amplitude of extensor motoneuron bursts (Conway *et al.* 1987; Pearson and Collins 1993).

Golgi tendon organs can also modulate the duration or timing of motor activity during walking. Electrical stimulation of tendon organ afferents increases the durations of the stance phase (intact and decerebrate cats), (Whelan and Pearson 1997a; Whelan and Pearson 1997b); decerebrate rats, (Fouad and Pearson 1997). Similar effects were elicited by stimulation of nerves (exciting both Golgi tendon organs and muscle spindles) in preparations showing fictive locomotion. [Fictive locomotion occurs as rhythmic activities in motor nerves that can be elicited by application of neurotransmitters or by brainstem stimulation in preparations that are paralyzed.] The same stimulation also caused a synchronous bursting in other supportive leg muscles (Pearson and Collins 1993). This pattern was shown to result from the specific activation of Golgi tendon organs, as the effects were not elicited when 1a afferents from muscle spindles were selectively stimulated by vibration.

Discharges of muscle spindles may also affect the magnitude and timing of motor activities. Sensory recordings have shown that muscle spindles of leg extensors are active during the stance phase (Pearson *et al.* 1998). In fictive locomotion, selective activation of ankle extensor spindles by muscle stretch can excite other leg extensor muscles (Guertin *et al.* 1995). This experiment also showed that stimulation of spindles led to a delay in the onset of the flexor bursting (initiation of swing).

The influence of cutaneous inputs on walking was investigated by electrically stimulating the sural nerve in freely walking humans (Duysens *et al.* 1996). Stimulation of the nerve has multiple effects depending on the phase (swing or stance) in which the stimulus is applied. If the nerve is stimulated during the first part of stance, the effect is excitatory to supportive extensor muscles. Changes in motor timing are also observed when cutaneous afferents are stimulated. However, the potential for affecting the transition from stance to swing by cutaneous input from the foot is strongly dependent on the presence of other sensory inputs (Duysens *et al.* 2000).

Thus, a number of types of sense organs of vertebrates can enhance activities of leg extensor muscles that act to counter body load. This conclusion is supported by studies that have been performed on cats in which all sensory inputs from a single leg were lesioned by cutting dorsal roots. These animals showed large deficiencies in the generation of the walking rhythm (Wetzel *et al.* 1976). The deafferentated leg was unable to display extended stance phases, which are necessary for supporting the body load during locomotion. Experiments in this thesis will also study the effects of removal of sensory inputs in cockroach walking, which can produce similar effects to those seen in cats.

In invertebrates, sense organs that detect load can modulate the magnitude and timing of motor outputs in ways that are remarkably similar to vertebrates. Cuticular mechanoreceptors of the crab dactyl and the campaniform sensilla of insects have been particularly well studied and shown to influence motor responses in much the same way as the Golgi tendon organs in vertebrates. Bässler demonstrated that tonic stimulation of campaniform sensilla of the trochanter in stick insects (by means of a small attached clip) caused the leg to remain extended at all times during walking, presumably through tonic activation of extensor motoneurons (Bässler 1977). A similar stimulus applied to the trochanteral campaniform sensilla in restrained cockroaches produced vigorous excitation of the trochanteral extensor muscle (Pearson 1972). These effects are similar to the activation of leg extensors produced by stimulation of Golgi tendon organs in cats.

Force receptors can also modify the timing of walking movements. Receptors in the crab (Libersat *et al.* 1987a; Libersat *et al.* 1987b) and cockroach (Zill and Moran 1981) fire during stance and are silent in the swing phase. Electrical stimulation of receptors of the dactyl (foot) can reset the rhythm of walking movements and effect the timing of both stance and swing (Libersat *et al.* 1987a; Libersat *et al.* 1987b). Stimulation of the trochanteral campaniform sensilla delays the onset of flexor bursts (active in swing) in preparations with cut thoracic connectives (a form of fictive locomotion) (Pearson 1972). It has also been shown that swing phase duration is decreased by having cockroaches walk uphill (Pearson 1972).

Joint angle receptors can contribute to modulations of motor activities during walking in ways that are more complex than simple reflexes. Spirito et al. studied the effects of perturbations that produced deviations of leg joint angles during standing and walking in crabs. Perturbations applied in animals that were restrained (described above) produced excitation of specific muscles in resistance reflexes that opposed the joint movement (Spirito *et al.* 1972). Interestingly, this pattern did not occur to joint movements that were produced by the animal itself in unperturbed walking. However, imposition of perturbations during walking (by impeding joint movements with a solenoid attached to the leg) produced strong resistance discharges. It was hypothesized that resistance reflexes do not initiate or drive locomotor activity in walking, but rather are modulated so that they occur only to unexpected changes in leg position.

Joint angle receptors can also determine timing of motor bursting during walking (Bässler and Büschges 1998). The stance to swing transition is influenced by two main variables in both vertebrates and invertebrates. First, the leg must be unloaded to enter the swing phase. Secondly, a leg will be more likely to enter swing if it is fully extended. In stick insects, it has been shown that loads that resist leg extension decrease the velocity of movement and increase the step period (Dean 1991). The increased duration of the stance phase results in a delay in the onset of swing.

Inputs from chordotonal organs may be important in generating support during the stance phase and in the initiation of swing. A reflex-like influence called the "active reaction" has been described that can aid in determining the end of stance and initiation of swing in stick insects (Bässler and Büschges 1998). In active animals, displacements of the femoral chordotonal organ, that mimic joint movements in walking, do not produce resistance reflexes. Instead these displacements elicit consistent activation of stance phase muscles. However, swing phase muscles become active when the displacement is sufficient and the organ indicates the position at the end of stance (Bässler and Büschges 1998). Thus, the active reaction may serve to delay the onset of swing until a leg is in the appropriate position.

Swing to stance transitions in insects can be influenced by ground contact. However, it was shown that animals were not prevented from entering stance by selective ablation of the tarsus (foot) (Duysens *et al.* 2000). Therefore, as in vertebrates, it is likely that other receptors such as position or force indicators are more essential to initiation of stance.

Conclusions

This introduction has shown that the detection of load can have significant effects upon motor activities generated by the nervous system in both standing and walking. A consistent finding in both vertebrates and invertebrates is that changes in load can affect both the timing and magnitude (motor frequency and amplitude) of motor bursts. The following chapters in this thesis will describe experiments that attempt to determine how campaniform sensilla of the American cockroach detect the effects of body load during standing and walking. They will also examine how the sensilla effects motor activities in posture and locomotion. The first two studies will ask the questions: 1) Do the tibial campaniform sensilla encode the effects of body load? and 2) How are discharges of the receptors altered when body load is reduced? The final study will examine how walking patterns are altered by leg ablations that selectively eliminate sensory inputs.

CHAPTER 1

SENSING THE EFFECT OF BODY LOAD IN LEGS: RESPONSES OF TIBIAL CAMPANIFORM SENSILLA TO FORCES APPLIED TO THE THORAX IN FREELY STANDING COCKROACHES

SUMMARY

Sense organs in the legs that detect body weight are an important component in the regulation of posture and locomotion. We tested the abilities of tibial campaniform sensilla, receptors that can monitor forces in the cockroach leg, to encode variations in body load in freely standing animals. Small magnets were attached to the thorax and currents were applied to a coil below the substrate. Sensory and motor activities were monitored neurographically. The tibial sensilla could show vigorous discharges to changing forces when animals stood upon their legs and actively supported the body weight. Firing of individual afferents depended upon the orientation of the receptor’s cuticular cap: proximal sensilla (oriented perpendicular to the leg axis) discharged to force increases while distal receptors (parallel to the leg) fired to decreasing forces. Proximal sensillum discharges were prolonged and could encode the level of load when increases were sustained. Firing of the trochanteral extensor motoneuron (Ds) was also strongly modulated by changing load. In some postures, sensillum discharges paralleled changes in motor frequency consistent with a known interjoint reflex. These findings demonstrate that tibial campaniform sensilla can monitor the effects of body weight upon the legs and may aid in generating support of body load.

INTRODUCTION

Support of the weight of the body is an essential requirement for standing, walking and running (Horak and Macpherson 1996; Duysens et al. 2000). The head, thorax and abdomen form the majority of body mass in most animals and must be elevated above the substrate for efficient locomotion (Puniello et al. 2000; Kram and Full 1997). However, the effects of body weight upon the legs are not constant but change according to behavior of the animal. In standing, the extent of body load supported by a leg depends upon its position relative to the center of mass (or pressure) (Full et al. 1991) and upon the number of legs being used in active support (Pratt 1995). In walking, ongoing variations in loading of individual legs occur in the cycles of stance and swing (Chang et al. 2000). Sudden changes in the effect of body weight and inertia must be rapidly compensated when a leg loses footing and slips (Duysens et al. 2000; McIlroy and Maki 1993). Tonic changes in body load can also occur when carrying objects (weights, food) or in child bearing (Kram 1996).

In most animals, body load is supported by an anatomically discrete set of skeletal and muscular elements (Alexander 1988). Passive support is provided by bones and ligaments while active compensation for load is generated by leg and body muscles. The limbs of many animals are designed, positioned and attached to maximize support by bones and ligaments (Maloiy et al. 1986). Some of these structures are composed of elastic materials and specifically situated to provide for energy storage and release (Neff et al. 2000). However, in most animals, changes in load are also countered by alterations in activities of leg and body muscles. Load compensation by active muscle contractions can occur in a variety of limb positions and body orientations, without anatomical constraints of passive elements (Jacobs and Macpherson 1996). This can facilitate adaptation of posture and locomotion when traversing diverse terrains (Smith and Carlson-Kuhta 1995).

Changes in the effects of body load upon the legs are thought to be detected predominantly by sensory receptors in the limbs (Prochazka 1996). Many types of leg sense organs (Vertebrates: Golgi tendon organs, muscle spindles, pressure receptors; Invertebrates: campaniform sensilla, funnel canal organs, apodeme tension receptors, chordotonal organs) can directly or indirectly encode effects of body load. Activation of some of these receptors can induce motor discharges that could aid in adjusting activities of leg muscles that counter load variations (Zill 1990, 1993). However, the specific contributions of receptors of different modalities to detection of body load have remained controversial. Discharges of afferents have only rarely been characterized in experiments examining the effects of changes in body load during posture and locomotion (Prochazka et al. 1977; Aniss et al. 1990).

We have studied the effects of changing body load while recording activities of the tibial campaniform sensilla, sense organs that monitor forces in the legs of insects (Pringle 1938). These receptors detect the direction, magnitude and rate of change of force increases or decreases in the limbs (Ridgel et al. 1999, 2000). The tibial sensilla consist of two subgroups (proximal and distal receptors) with mutually perpendicular orientations of their ovoid cuticular caps, the presumed sites of mechanotransduction (Spinola and Chapman 1975). These subgroups show differential sensitivities to forces imposed upon the leg and to resisted contractions of leg muscles (Zill and Moran

1981a). They also elicit different reflex effects in motoneurons to limb muscles (Zill and Moran 1981b).

The activities of the tibial sensilla can also be recorded in freely moving animals (Zill and Moran 1981b; Ridgel et al. 2001; Noah et al. 2001). The proximal receptors discharge tonically in upright posture. The proximal and distal sensilla show alternating bursts during the stance phase of walking. These discharges were first considered to result from leg loading and resisted contractions of the tibial extensor muscle, respectively. However, firing of the tibial sensilla was found to persist when animals walked with body weight supported on an oiled glass surface (Noah et al. 2001). The patterns of discharge of proximal sensilla were altered under these conditions: intense firing occurred immediately after the onset of stance but activity later in stance was substantially reduced as compared to animals walking freely and supporting body load. These findings were consistent with the following hypotheses: the early component of the discharges was considered to result from contractions of leg muscles initiated prior to leg contact that pressed the foot against the walking surface (Watson and Ritzmann 1998). The reduction in firing later in stance was thought to result from the fact that the effect of body weight upon the legs was reduced or eliminated when supported, although this was not directly tested. Sensitivity to body mass and inertia was also found in standing animals in studies using horizontal perturbations of the substrate (Ridgel et al. 2001). Tibial sensilla fired at short latency to horizontal displacements consistent with their directional sensitivity. However, more complex discharges occurred in tests in which the substrate was displaced at higher velocities or increased amplitudes. These discharges were thought to result from displacement of the body mass, particularly the abdomen, which is not directly supported by the legs. However, no direct tests were systematically performed in which body load was altered to test this hypothesis.

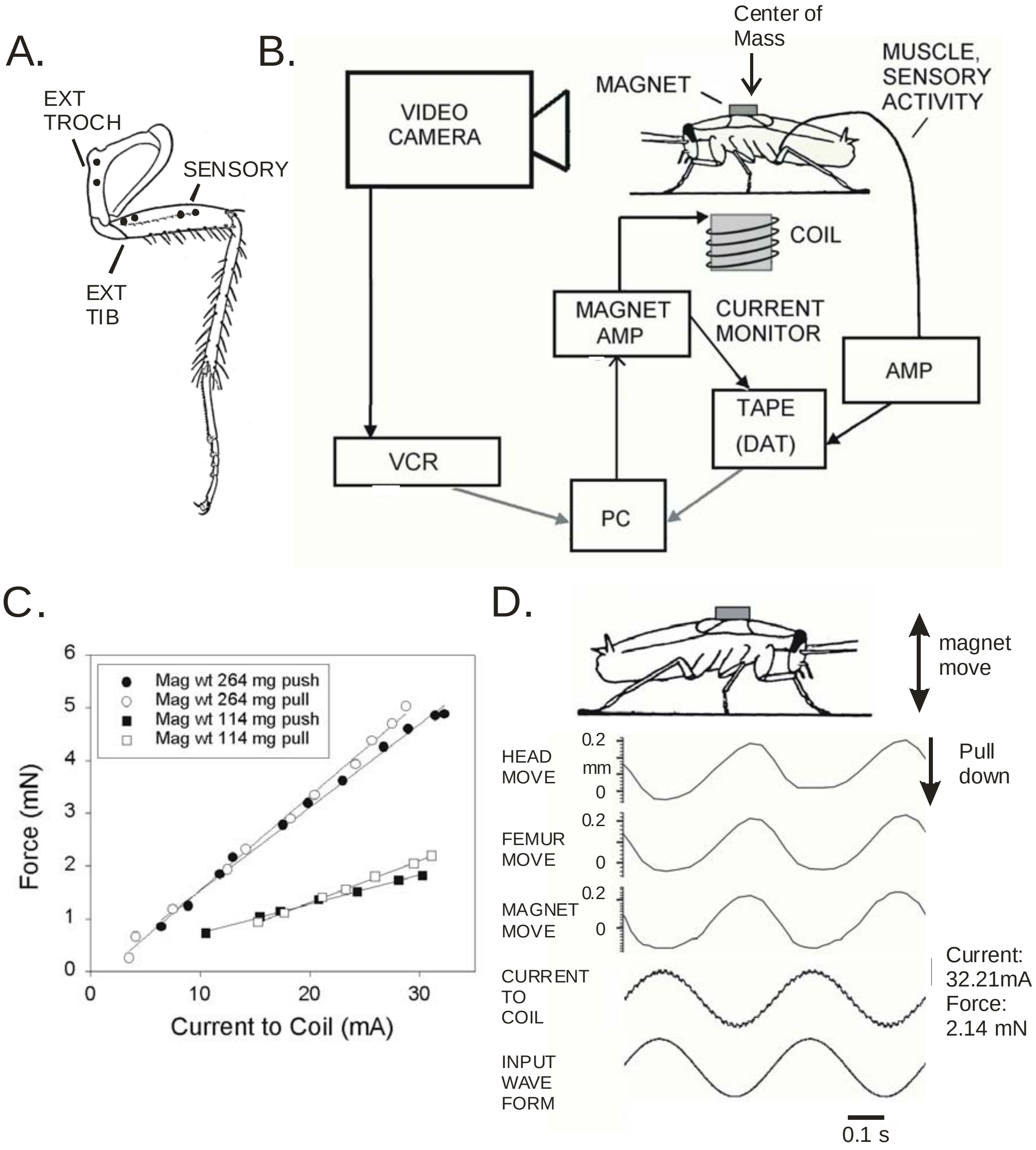
The present experiments sought to answer the questions: 1) can campaniform sensilla detect the effects of variations in body load in freely standing animals and 2) could information from the receptors be incorporated into load compensatory responses? We have studied responses to changes in load by experimentally varying forces that act on the legs through small magnets attached to the animal's body. These experiments demonstrate that the tibial campaniform sensilla can readily detect the effects of increases and decreases in body weight upon the legs. The tonic component of discharges of the proximal sensilla can also reflect the extent of leg loading. They also suggest that inputs from the receptors may complement information from other afferents, such as joint angle receptors, and contribute to compensatory reactions to increases in load.

METHODS

Experimental setup

Cockroaches (Periplaneta Americana males obtained from Carolina Biological Supply) were first restrained and fine wires were implanted into the leg to record activities of the tibial group of campaniform sensilla (n=22 animals) (Fig. 1A) (Zill and Moran 1981a; Ridgel et al. 1999). In some experiments, two pairs of sensory recording leads were utilized (Ridgel et al. 2001), while in other animals myographic leads were inserted in the trochanteral or tibial extensor muscles (11 animals) (Watson and Ritzmann 1998). In most experiments, a thread was then tied as a suture through holes

Figure 1: Preparation for changing forces using magnetic fields. A. Neurophysiological recordings - Fine wire electrodes were placed into the left hindleg of cockroaches to record sensory and motor activities (SENSORY = nerve 5r8, EXT. TIB. = tibial extensor muscle, EXT. TROCH. = trochanteral extensor muscle). B. Apparatus – Magnets (MAGNET) were attached to the dorsum of the animal, either directly or by means of a metal harness, at a point immediately anterior to the center of mass. Preparations were placed into an arena above a magnetic coil (COIL). Currents to the coil were generated by computer (PC) and amplified (MAGNET AMP) and acted to pull or push the animal towards or away from the substrate. Preparations were videotaped by a high-speed camera (VIDEO CAMERA, VCR) and video data were synchronized with electrical recordings as in Noah et al. 2001. Neurophysiological data (AMP) and coil current (CURRENT MONITOR) were recorded on tape (TAPE DAT). C. Calibration - The vertical force produced by the action of the coil was calibrated by attaching magnets to a single axis force plate at a height equivalent to that used in experiments. When current was passed through the coil, the magnets were pulled down or pushed up. The force developed was linearly related to the magnitude of the current for each group of magnets. D. Movements - Vertical displacement of the magnet and points on the head and femur were measured from video images of a freely standing animal and synchronized with recordings of input waveform and current to the coil. Small shifts in position of the magnet and body markers occurred in fixed relation to sinusoidal currents delivered to the coil. The height above the substrate decreased during the rising phase of the sine wave and increased in the opposite phase.


A.
EXT TROCH
SENSORY
EXT TIB
B.
Center of Mass
MAGNET
MUSCLE, SENSORY ACTIVITY
VIDEO CAMERA
COIL
MAGNET AMP
CURRENT MONITOR
AMP
TAPE (DAT)
VCR
PC
C.
Mag wt 264 mg push
Mag wt 264 mg pull
Mag wt 114 mg push
Mag wt 114 mg pull
Force (mN)
0
1
2
3
4
5
6
0
10
20
30
Current to Coil (mA)
D.
magnet move
HEAD MOVE
0.2
mm
0
Pull down
FEMUR MOVE
0.2
0
MAGNET MOVE
0.2
0
CURRENT TO COIL
Current: 32.21mA
Force: 2.14 mN
INPUT WAVE FORM
0.1 s

made in the base of the wings at the point of their attachment to the thorax. A small square of tape, covered with a layer of metal dust (Steel of West Virginia, Inc.) and cyanoacrylate adhesive, was firmly glued to the suture and body to form a rigid base (Noah et al. 2001). In other studies, a small neodymium magnet (weight = 57mg, Indigo Instruments, Waterloo, ON) was glued directly to the wing base, and the wings were glued to each other using a small drop of adhesive. The latter method assured that the orientation of the magnets was parallel with the wing base. In most experiments, small pairs of dots were painted on the coxal, femoral and tibial segments of the legs using white nail polish as markers.

Animals were then released into an arena that had a plexiglass or fine nylon mesh floor (Fig. 1B). A single or pair of neodymium magnets was placed upon the tape (or small magnet) producing a tonic increase in the weight of the animal. The field strength of the magnets was sufficient to adhere to the animal with no further adhesives. However, they could be readily removed by the experimenter with a pair of forceps. The position of the magnets was generally directly over the middle leg coxae. This point is somewhat anterior to the center of mass (estimated to be the anterior margin of the hindleg coxa by balancing bodies of animals that were frozen (Kram and Full 1997)). However, this location was found to be more stable and prevented the animal from tilting toward the abdomen when current was applied with the hindlegs fully flexed. An electrical coil (Dura Magnetics, Sylvania OH) was mounted on a moveable support below the arena so that it could be positioned directly beneath the magnet. Currents to the coil were produced by a waveform generator or by a computer interface (Cambridge Electronics) and amplified (DC Power Amplifier Model 124, APS

Dynamics). These currents generated magnetic fields in the coil that attracted or repelled the magnet on the dorsum of the animal, which effectively varied the forces acting upon the legs.

Protocol

Perturbations were applied when animals assumed quiet postures and stood on the floor of the arena. The magnet coil was rapidly positioned under the animal. A marker on the coil, indicating the position in which the magnets were displaced vertically (see Calibration below), was moved directly below the magnet on the animal. Sinusoidal currents were produced by a waveform generator and the frequency varied by hand. Half sine waveforms and ramp and hold functions were generated by computer. The half sine waveforms rapidly rose from zero voltage and then returned. The ramp and hold waveforms were applied as follows: the current first gradually went to a negative value and held for 1 second, then rose to an equivalent positive value and held for 1-2 seconds, then changed back to the unloaded value. This produced a sequence of loading and unloading that was repeated five times in a trial. The current then returned to zero for a rest period. The pattern was reversed in other experiments by first going to a loaded value and then rapidly unloading the animal. Most animals did not hold single positions for long periods and gross movements of the legs or body were observed. Movements were also elicited if the animal was pulled down to an extent that the abdomen touched the substrate. In these events, there were abrupt changes in sensory and motor firing frequencies. However, it was possible to record some sequences in which animals held positions for long periods.

Animals could readily counter the additional load but in some sequences, tilting of the body could be observed, depending upon the position of the legs. The general patterns of sensory and motor responses to force increases reported in this paper occurred when the body tilted toward the side of the recording, indicating an increased load on that side. Sequences in which extreme tilting occurred were not analyzed. In addition, the attachment of the magnets to the wing base potentially introduced some compliance or viscoelasticity in coupling to the body. However, we found that the sensilla responded at very short latency to the stimulus, indicating that forces producing leg loading were transmitted very rapidly.

Measurements of body movements produced by the coil - Movements of the body and legs were monitored at extreme close up by a high-speed video camera (Redlake Motionscope, 125 or 250 frames/second) through the side of the arena. The camera had sufficient memory for the final 8 or 16 seconds of a sequence (depending upon the frame rate) to be downloaded to videotape at 30 frames/sec. We digitized the position of the magnet or dots on the body from images as a measure of body movement, as points on the ventral surface of the animal were too close to the substrate to be clearly visible in all images (see Cruse et al. 1989 for techniques in free standing stick insects). This gave a measure of the change in position of the body, which is only an indirect indicator of the change in body height. However, it did give us a measure of when sensory discharges were initiated, relative to any changes in position.

Neurophysiological recordings, magnet currents and voltages were stored on digital tape (TEAC) for later transcription and analysis. Video data were synchronized with the neurophysiological recordings as described elsewhere (Ridgel et al. 2001; Noah et al. 2001).

Calibration for level of force exerted

We first monitored the effects of the coil upon magnets that were supported by flexible mounts at distances equivalent to those used in tests of imposed forces. The effect of the coil depended upon the location of the magnet in its field, but the coil produced vertical displacements without rotation or lateral movements when appropriately positioned. This point was marked on the coil and positioned under the animal during load tests. We also measured the vertical force levels exerted by the coil by using a single axis force plate (that had strain gauges attached to its upper and lower sides). The magnets were placed upon the plate and the coil was positioned at a height equivalent to that used in experiments. Currents were applied using the ramp and hold waveforms that were utilized in tests of static loading. The forces exerted upon the magnet by the coil are plotted in Figure 1C for different weights of attached magnets. The static force levels exerted by the coil were linearly related to the applied current and forces exerted by positive currents (pulling the magnet toward the substrate) were approximately equivalent to those for negative currents (pushing the magnet away from the floor of the arena). Linear regression equations were calculated from these calibrations and used to estimate the forces exerted upon the animal in load experiments.

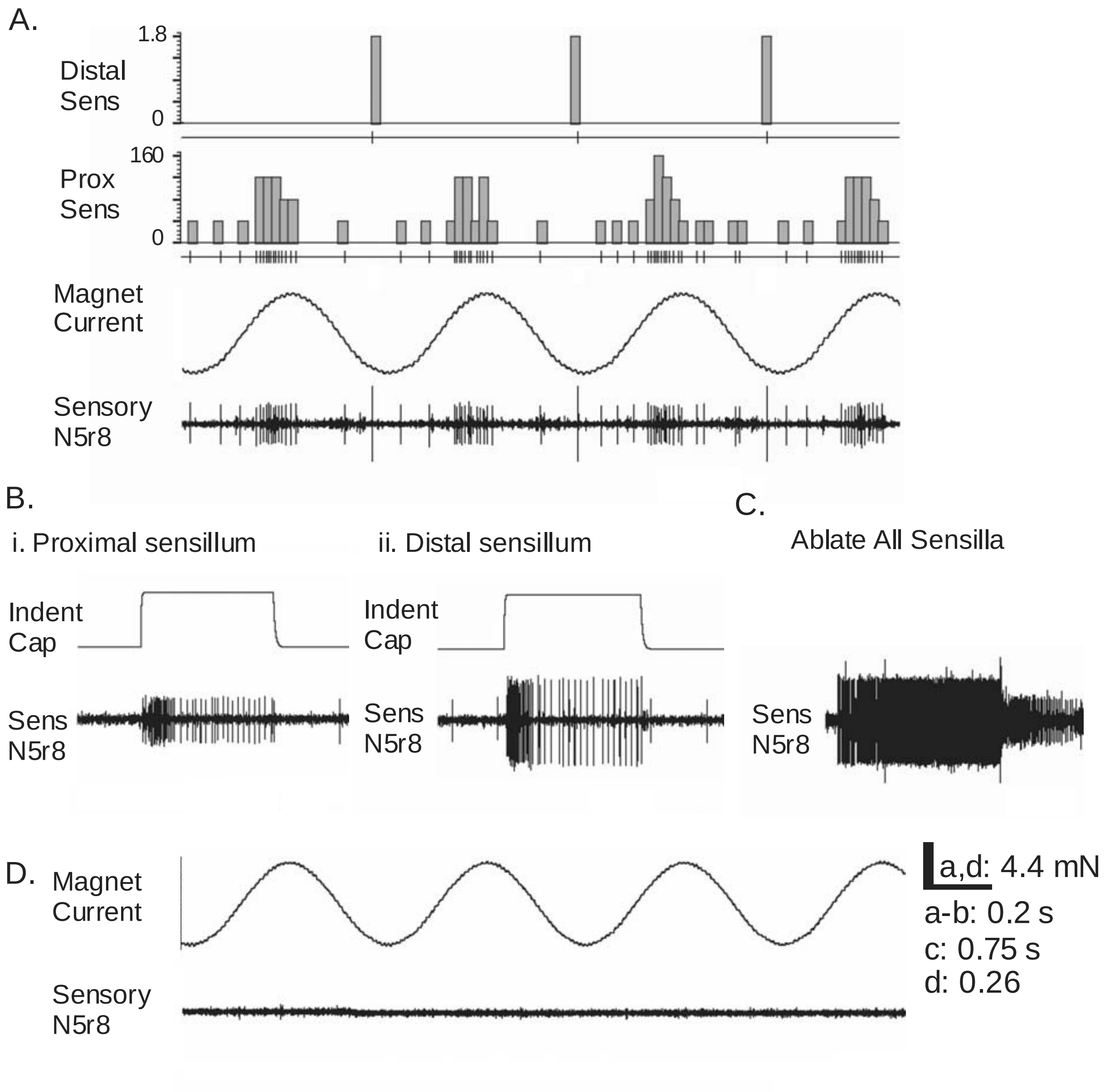
Furthermore, activation of the coil alone, without the magnet being placed upon the animal's dorsum produced no change in motor or sensory activities. In addition, no sensory or motor responses were elicited unless the animal elevated the body from the substrate, as evidenced by lifting of the thorax and abdomen in video images. These observations confirmed that the effects of the magnet fields were limited to the mechanical forces they produced.

RESULTS

Sensory responses to sinusoidal variations in load

Currents applied to the coil could produce bursts of activity in the tibial campaniform sensilla that were phase linked to the stimulus, when animals stood with the thorax raised from the substrate and supported by the legs. Figure 2A shows a recording from nerve 5r8 in which a continuous sinusoidal waveform (frequency 1.7 Hz, current 26 mA) was applied to the coil below a freely standing animal that had magnets (net weight 264 mg) attached to the dorsal side of the thorax. This resulted in force upon the animal that had an estimated maximum increase and decrease of +/- 4.4 mN, based upon regression equations calculated from the calibration in Figure 1C. The proximal sensillum fired a burst of spikes during the rising phase of the sine wave in each cycle, when forces were developed that pulled the magnet toward the substrate. This discharge ceased abruptly or rapidly declined when the sine wave reached a maximum. A distal receptor fired a single action potential during the opposite phase of each cycle, when the weight upon the legs decreased. The identification of units in the sensory recording was confirmed by restraining animals after initial tests in the arena. Figure 2B shows discharges of the receptors that were obtained by indentation of individual cuticular caps of proximal (i) and distal (ii) sensilla with a fine wire probe. The size of the extracellularly recorded action potentials of the sensilla correspond in amplitude to the units recorded during the test in Fig. 2A. Ablation of the cuticular caps

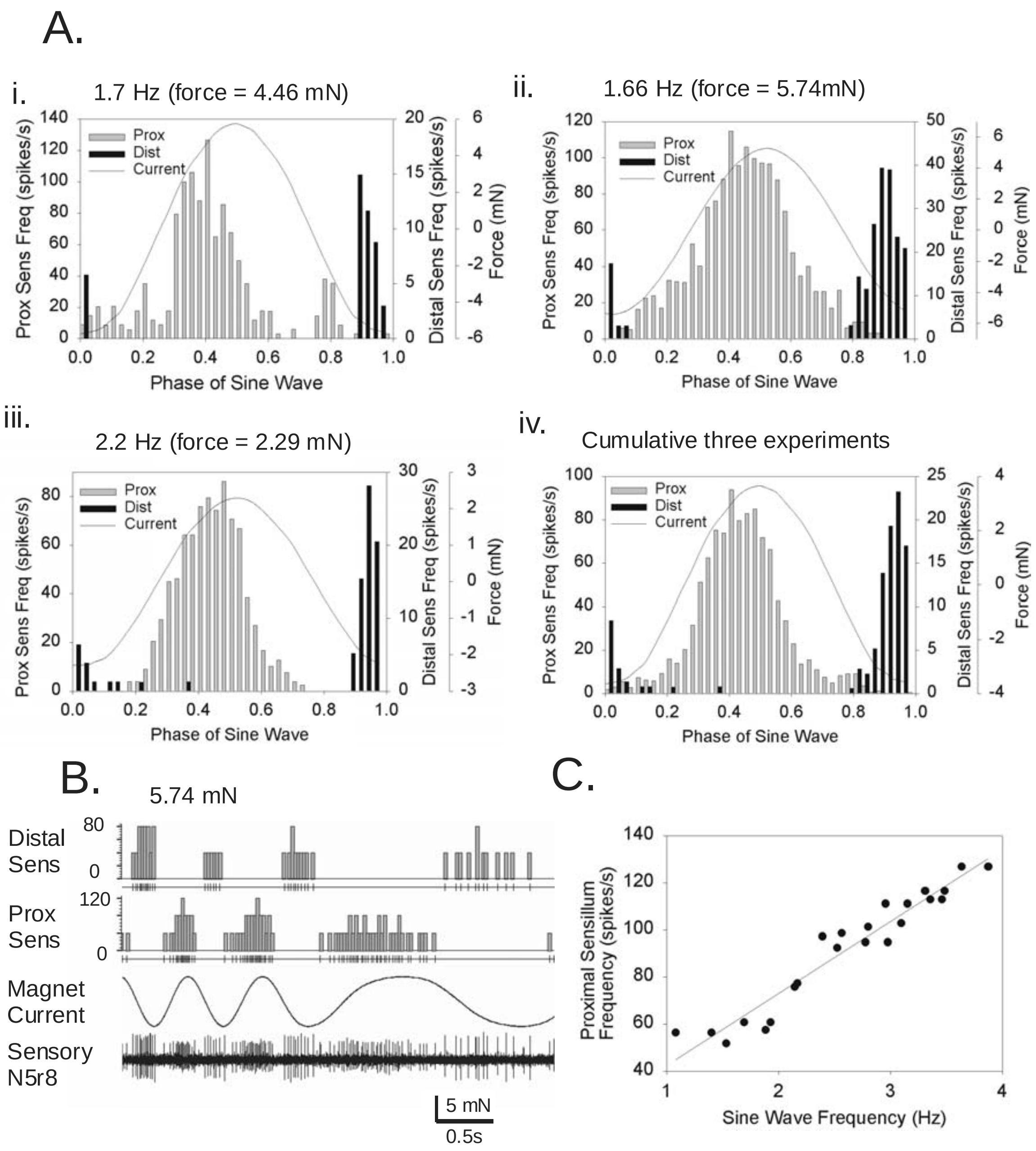
Figure 2. Sensory activities during sine wave variation in load A. Recordings of the tibial campaniform sensilla (Sensory nerve 5r8) during application of sine wave current to the coil (Magnet current) while the animal maintained a posture standing on its legs. The proximal sensilla (Prox Sens) fired bursts during the positive phase of the current, when the animal was pulled towards the substrate. The distal sensilla (Distal Sens) fired single spikes during the negative phase, when the animal was pushed away from the floor. B,C. Controls for sensillum identification – The animal was restrained after the initial sine wave test. Indentation of the caps (Indent Caps) of individual proximal and distal sensilla (B) produced discharges in the sensory recording (Sens N5r8) that matched the size of units seen in A. Ablation of the caps (C) produced injury discharges of similar amplitude. D. Re-test responses to load – Cap ablation eliminated all large unit activities when animals were released and responses were re-tested.


A.
1.8
Distal
Sens
0
160
Prox
Sens
0
Magnet
Current
Sensory
N5r8
B.
i. Proximal sensillum
Indent
Cap
Sens
N5r8
ii. Distal sensillum
Indent
Cap
Sens
N5r8
C.
Ablate All Sensilla
Sens
N5r8
D.
Magnet
Current
Sensory
N5r8
a,d: 4.4 mN
a-b: 0.2 s
c: 0.75 s
d: 0.26

and receptors (Fig. 2C) produced injury discharges of similar spike size. These ablations eliminated all large units in the recording, when animals were returned to the test arena and responses to changes in load were re-tested in a number of postures assumed by the animal (Fig. 2D).

In all animals, the responses of individual sensilla exhibited consistent directional sensitivity according to the orientation of the receptor's cuticular caps. Figure 3A shows cumulative histograms of the frequency of discharge of proximal and distal sensilla vs. the phase of the sinusoidal current in which they occurred (the cycles were divided into 40 bins). The data in Figs. 3Ai-Aiii are derived from single tests in which animals assumed and held postures over repeated cycles of load change. Fig. 3Ai is a plot of the discharge obtained during the test from which the recording in Fig. 2A is derived (24 cycles at 1.7 Hz, force +/-4.45 mN). Figures 3Aii and 3Aiii are histograms from sinusoidal tests applied in other animals (3Aii 22 cycles at 1.7 Hz force +/- 5.7 mN; 3Aiii 67 cycles at 2.2 Hz force +/- 2.2 mN). Figure 3Aiv is a composite histogram of the average of all three tests. In each individual case and in the cumulative histogram, the responses of individual sensilla showed increases in discharge rate or discrete bursts in similar phases of the sine wave stimulus. Proximal sensillum firing occurred as bursts or accelerations in the rising phase of the sine wave, as the forces changed from pushing the animal away from the substrate to pulling it down toward the floor. The firing frequency accelerated rapidly and reached a maximum near the peak of the sine wave. The peak firing frequency that was attained was generally dependent upon the level of force that was applied. Subsequently, proximal firing did not strictly follow the force amplitude but showed considerable hysteresis and declined rapidly after the peak

Figure 3. A. Cumulative histograms of sensory discharges in repeated cycles of sine wave changes in load. Cycles of sine wave current were divided into 40 bins (from the minimum voltage value). The averaged discharge frequencies of proximal and distal sensilla were calculated and plotted with the mean sine waveform. The estimated force scale at right is based upon regression equations calculated from the calibration data in Fig. 1C (increased load is positive, decreased load is negative). 3Ai-iii are plots of the discharges from tests in three different animals, 3Aiv is a composite of the average of these tests. In each individual trial and in the cumulative histogram, proximal firing accelerated above baseline during the rising phase of the current but showed hysteresis and fell rapidly following the peak of pulling force. Distal sensilla did not fire until near the maximal unloading force. B. Recording of sensory and motor activities as the rate of sine wave current is slowed. Firing frequencies of proximal and distal sensilla declined with decreased rate of change of force. Distal sensillum firing was particularly robust in this preparation. C. Plot of maximum discharge rate of proximal sensillum in preparation that held a posture through a range of sine wave frequencies - Proximal sensillum encoded the rate of change of forces while holding a single posture (Rsquared value = 0.93).


A.
i.
1.7 Hz (force = 4.46 mN)
ii.
1.66 Hz (force = 5.74mN)
iii.
2.2 Hz (force = 2.29 mN)
iv.
Cumulative three experiments
Prox
Dist
Current
Prox Sens Freq (spikes/s)
Distal Sens Freq (spikes/s)
Force (mN)
Phase of Sine Wave
0.0
0.2
0.4
0.6
0.8
1.0
B.
5.74 mN
Distal Sens
80
0
Prox Sens
120
0
Magnet Current
Sensory N5r8
5 mN
0.5s
C.
Proximal Sensillum Frequency (spikes/s)
Sine Wave Frequency (Hz)
140
120
100
80
60
40
1
2
3
4

of the sine wave, even though the effective body weight was still increased. The extent of the hysteresis and detailed pattern of excitation varied and inhibition was less complete when background levels of sensillum firing were high (Fig. 3Aii).

Distal sensilla discharged during the phase in which the magnet was pushed away from the substrate. There was generally little overlap in the phase in which distal and proximal receptors were excited and co-activation of sensilla of different orientations was rarely obtained (Zill and Moran 1981a). However, the onset of distal bursts most often did not occur until the sine wave approached its negative peak and was therefore delayed in phase relative to that seen in proximal receptors. Furthermore, distal firing was more variable than proximal discharges. In some cases, distal sensilla fired single spikes while in other cases large bursts occurred (compare Figs. 2A and 3B). Discharges of distal receptors also did not occur in all cycles, as is reflected in the lower mean rates of distal firing in the histograms. Some of this variability may result from the fact that the weight of the magnets produced a tonic loading of the animal and that unloading of a single leg was not complete.

Sensitivity to rate of stimulation

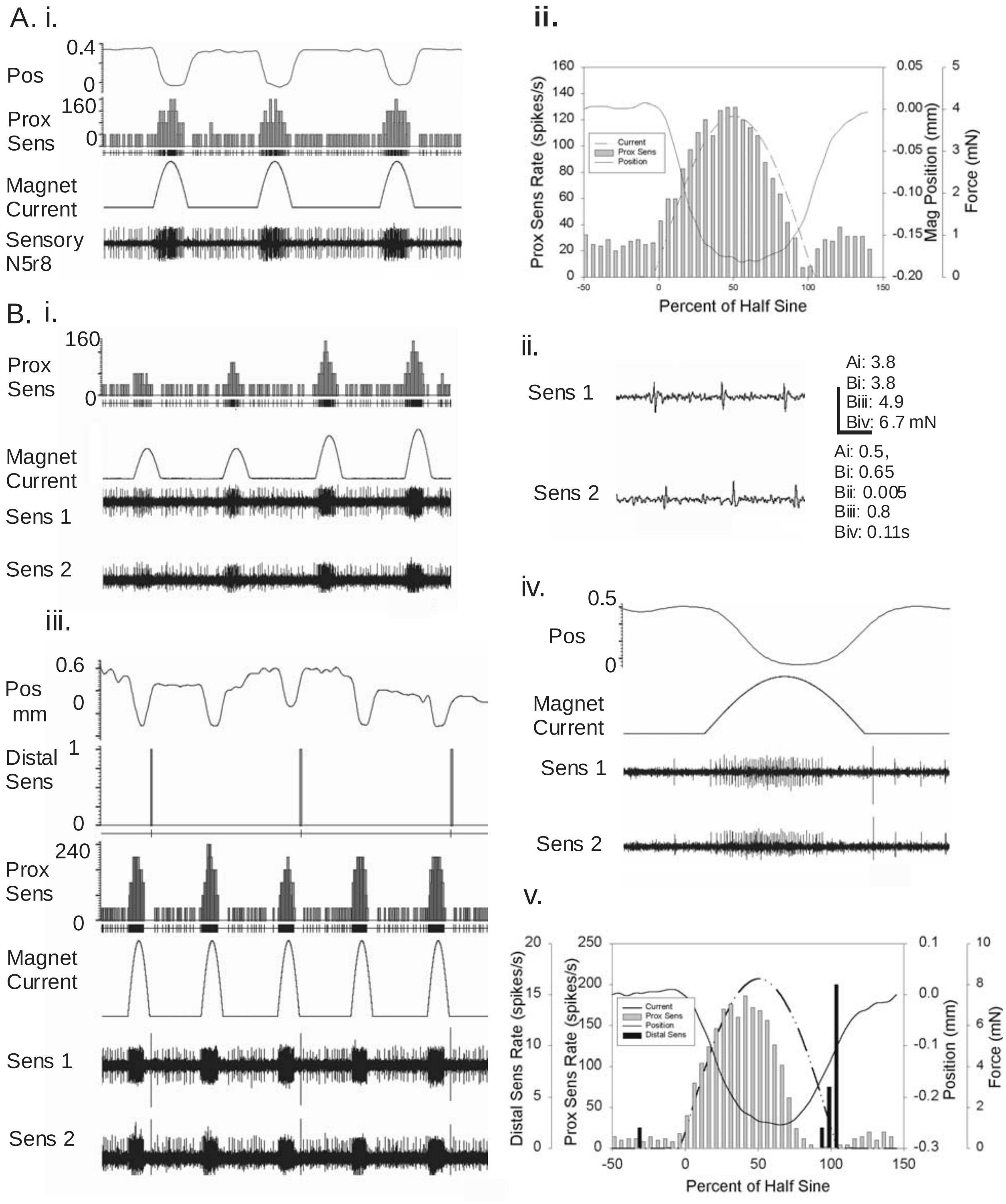
The firing frequencies of tibial sensilla showed a dependence upon the rate of sinusoidal stimulation in all preparations (Fig. 3B,C). However, animals often changed position or began walking if the rate of change of imposed force became too high. Figure 3B shows a sequence in which the rate of sinusoidal stimulation was decreased at the end of a test (this test is from the experiment plotted in Figure 3Aii and showed a particularly high level of distal firing). The maximum firing frequency of both proximal and distal sensilla decreased as the rate was slowed. Figure 3C is a plot of the maximum firing rate seen in a proximal sensillum for a sequence from another animal in which the rate was changed over 10 seconds and the preparation held the position despite the high rate of sinusoidal stimulation (force amplitude 2.51 mN). Over this range the sensitivity to the rate of current applied to the coil was linear (R squared = 0.93). These findings suggest that the proximal receptors can effectively signal the rate at which body load is applied to the leg.

Sensory responses to sudden application of loads

When one leg of an animal loses friction or contact with the substrate, the load supported by the remaining legs can suddenly increase (Pratt 1995). We tested the ability of the tibial sensilla to encode rapid changes in the effect of body weight upon the legs by applying currents to the coil using half sine waveforms. These stimuli produced sudden increments in load and then rapidly declined to the initial level, which was increased somewhat above the normal body weight by the mass of the magnets. Figure 4Ai shows a recording in which half sine waves were applied as a series (with 1 second rests in between). The change in position of the magnet is also shown, as measured from digitized images that were synchronized with the recording. The magnet is briefly pulled down toward the substrate, following the onset of the stimulus, and then returns to its initial position without apparent rebound. The proximal sensillum firing accelerates above baseline immediately following the onset of the perturbation, then declines rapidly and is briefly inhibited near the end of the stimulus. Figure 4Aii is a cumulative histogram of the sensillum responses and averaged position of the magnet from this sequence with the superimposed current waveform. The proximal sensillum firing increases in the first histogram bin following the stimulus onset (bin duration = 28

Figure 4. Responses to sudden load increases. A i. Activities of tibial campaniform sensilla (Sensory N5r8) recorded while loads were rapidly increased and decreased by applying currents to the coil (Magnet Current) using half sine wave form (0.5 s duration, max force = 3.83 mN). The relative position of the magnet (Pos) was monitored from video images and synchronized with the recording to indicate the vertical displacement of the animal. A proximal sensillum (Prox Sens) rapidly increases its discharge following the onset of the stimulus as the animal is pulled toward the substrate. ii. Histogram of averaged firing of the proximal sensillum from 34 applications of the half sine waveform in the sequence shown in (i). The histogram was constructed from the onset of the stimulus and includes a period equivalent to half the stimulus duration before and after current application. The mean magnet displacement is also plotted from data on the last 9 repetitions of the stimulus (taken at 250 fps and downloaded at 30 frames per second). Proximal firing increases immediately following the stimulus onset and reaches a peak before the maximum displacement. B. i. Proximal activity recorded at two sites (Sens 1 and 2) in the femur during half sine perturbations at different amplitudes. Firing reaches a peak and then rapidly declines at all stimulus levels. ii. Spikes appear earlier in the more distal recording site (Sens 1) than the proximal electrodes (Sens 2) consistent with centripetal conduction. iii. Proximal firing (Prox Sens) to large stimulus amplitudes is often followed by distal firing (Distal sens) near the end of the declining phase. The position trace shows no rebound after the stimulus despite small movements by the animal over the trials. iv. Expanded view of the trial from iii. Proximal and distal sensilla fire 1:1 in each recording and unit activities are apparent within the intense burst. v. Histogram of 20 repetitions of the stimulus in

iii. Proximal firing shows considerable hysteresis and distal spiking occurs before the displacement has returned to its original position, during the period in which forces are declining.


A. i.
Pos
0.4
0
Prox Sens
160
0
Magnet Current
Sensory N5r8
ii.
Prox Sens Rate (spikes/s)
Mag Position (mm)
Force (mN)
Current
Prox Sens
Position
Percent of Half Sine
B. i.
Prox Sens
160
0
Magnet Current
Sens 1
Sens 2
ii.
Sens 1
Sens 2
Ai: 3.8
Bi: 3.8
Biii: 4.9
Biv: 6.7 mN
Ai: 0.5,
Bi: 0.65
Bii: 0.005
Biii: 0.8
Biv: 0.11s
iii.
Pos mm
0.6
0
Distal Sens
1
0
Prox Sens
240
0
Magnet Current
Sens 1
Sens 2
iv.
Pos
0.5
0
Magnet Current
Sens 1
Sens 2
v.
Distal Sens Rate (spikes/s)
Prox Sens Rate (spikes/s)
Position (mm)
Force (mN)
Current
Prox Sens
Position
Distal Sens
Percent of Half Sine

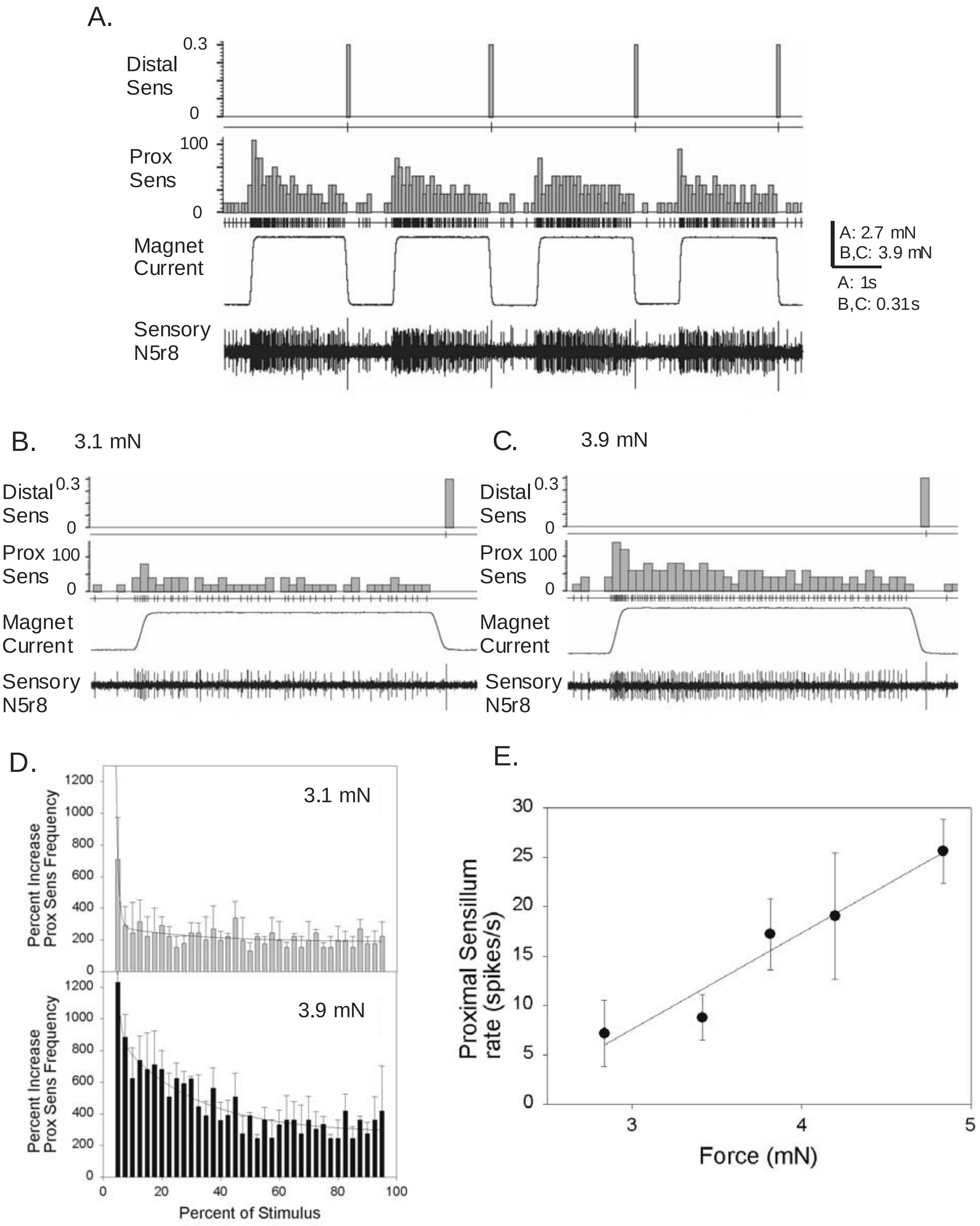
msec) and then rises in parallel to the current. The onset of increase in firing is approximately coincident with the start of the magnet movement Figure 4B is a similar series from another preparation in which the stimulus was applied with progressively increasing amplitude (at a rate of .568 Hz). The proximal sensillum firing accelerates at all levels and the maximum afferent frequency reflects the amplitude of the stimulus (Fig. 4Bi). Spiking activity of the receptor is visible in the dual recording and action potentials occur at a latency consistent with previous measurements of the conduction velocities of proximal sensilla (Fig. 4Bii). Figure 4Biii shows responses of the proximal receptor of the same experiment to currents of large amplitude (peak 8.29 mN) and also includes the trace of magnet position. At high levels of force application, the proximal sensillum fired an intense burst during the rising phase of the stimulus and the distal receptor discharged near the end of the declining phase, as forces returned to the initial level of load. Figure 4Biv shows an expanded segment of the recording in Fig. 4Biii while Figure 4Bv is a histogram comparable to Figure 4Aii for 20 repetitions of the stimulus (with the last 10 repetitions of the magnet position). The sensillum firing reached a peak before the maximum current and then declined very rapidly and ceased while the level of force was still considerably above baseline. This hysteresis is similar to that seen in the experiments with full sinusoidal waveforms.

In this sequence, the distal sensillum did not fire upon every repetition of the stimulus (Fig. 4Biii) and the distal spike often occurred at the end of or immediately following the half sine wave of current. The movement trace shows that this firing was probably not the result of rebound in the magnet position but instead occurred as the magnet slowly returned to its original position. Thus, the distal firing appears to be associated with a decline in the level of force (Ridgel et al. 1999), although the cause of the dampening of the return is unclear. In the present experiments, half sine stimuli were systematically applied only in the direction to pull the animal toward the substrate and transiently increase weight, then return the animal to the initial level. We found that reversing the stimulus to generate sudden unloading was often disruptive and led to movements by the animal to grasp the mesh floor.

Sensory responses to sustained loads

Legs of cockroaches can undergo sustained changes in load, as when standing on irregular terrains or when other limbs are lifted in grooming or in the swing phase of walking (Noah et al. 2001). We tested the responses of tibial sensilla to sustained changes in the effect of body weight using ramp and hold waveforms that alternately unloaded and loaded the animal. Discharges of proximal tibial sensilla were prolonged following the onset of tonic increases in load and could be sustained for the duration of the stimulus. Figure 5A shows a recording from a sequence in which the animal was alternately loaded and unloaded while it held a single posture. The proximal sensillum fires intense bursts that are sustained for the entire duration of the stimulus. A distal sensillum fires a single action potential upon unloading from the increased weight in each test. Figure 5B and C show single tests from the same sequence during sustained loading at two levels (3.1 and 3.9 mN). The proximal sensillum discharges phasico-tonically, with an initial high rate of firing that is initiated immediately after the onset of the stimulus and that adapts to a lower frequency over time. The distal sensillum fires a single spike close to the time of maximal unloading. The time course of adaptation of the proximal sensillum is plotted in Figure 5D. The initial phasic component of the

Figure 5. Responses to tonic loads. A. Activities of tibial campaniform sensilla during ramp and hold stimuli that produced alternate loading and unloading. The proximal sensillum fires an intense phasico-tonic discharge to prolonged loading. The distal sensillum fires a single action potential as the leg is unloaded. B. and C. Tonic loading at two different levels. The phasic component adapts rapidly at low levels of load, but adaptation is slower with larger loads. D. Histogram of mean responses from repetition of tests in B and C (low = mean of 10 tests, high = mean of 8 tests) all held in same position. Adaptation at both levels was best fit by curves with pairs of exponents (rapid Rsqr = .79; slow Rsqr = .879). This is potentially indicative of separate rapidly and more slowly adapting components. E. Plot of mean discharge of a proximal sensillum from a different preparation during the last 25% of the hold phase of the increase force. Each point is the mean of five repetitions at the same level of load increase. This preparation held the posture for a long period and the plot shows that tonic change in loading is encoded after initial adaptation.


A.
Distal Sens
0.3
0
Prox Sens
100
0
Magnet Current
Sensory N5r8
A: 2.7 mN
B,C: 3.9 mN
A: 1s
B,C: 0.31s
B.
3.1 mN
Distal Sens
0.3
0
Prox Sens
100
0
Magnet Current
Sensory N5r8
C.
3.9 mN
Distal Sens
0.3
0
Prox Sens
100
0
Magnet Current
Sensory N5r8
D.
Percent Increase Prox Sens Frequency
3.1 mN
3.9 mN
1200
1000
800
600
400
200
0
0
20
40
60
80
100
Percent of Stimulus
E.
Proximal Sensillum rate (spikes/s)
30
25
20
15
10
5
0
3
4
5
Force (mN)

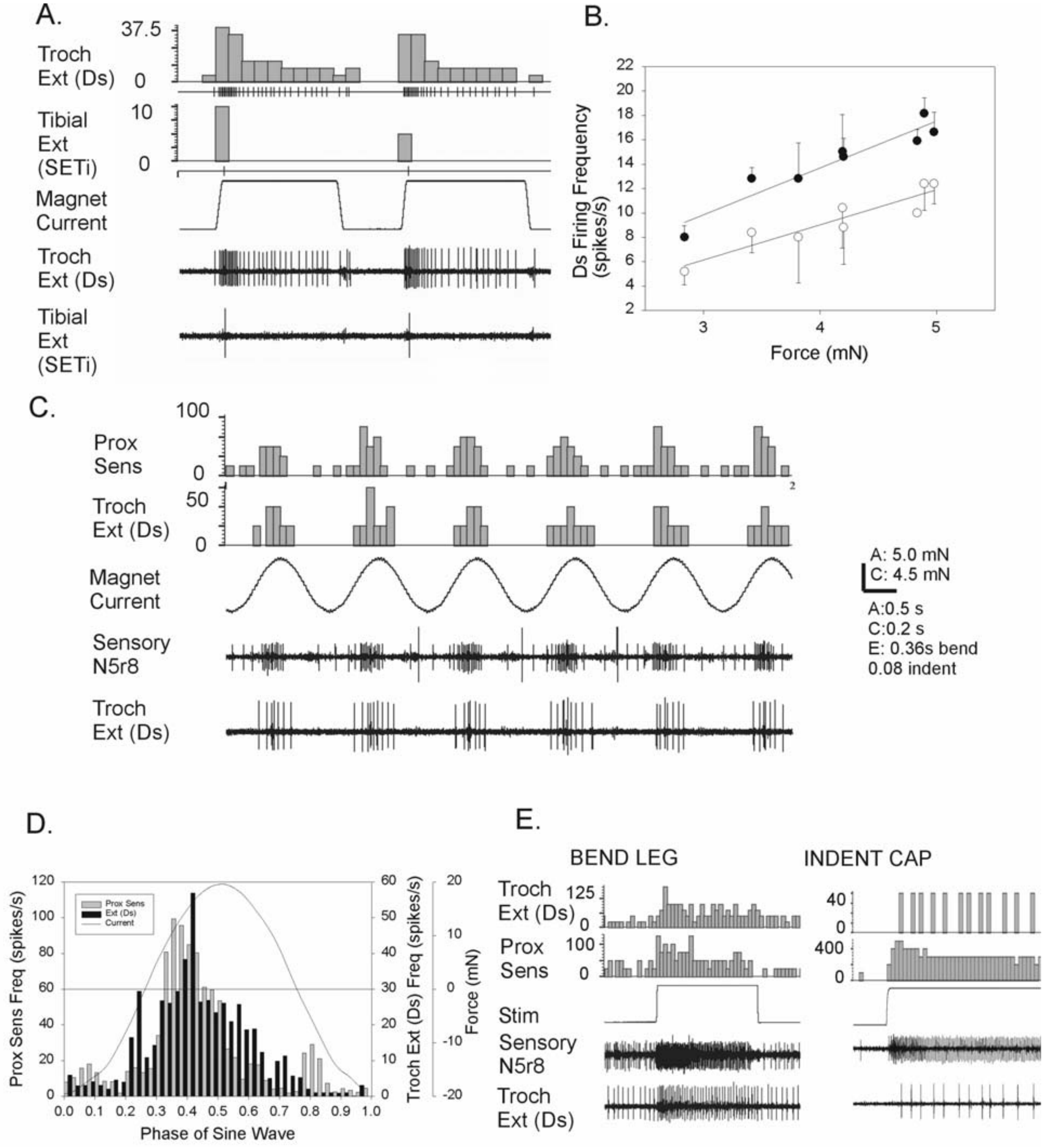
discharge is very brief at low levels of force and rapidly adapts to a level that is increased above baseline by 200%. When larger forces are applied, adaptation is more prolonged but within one second afferent firing reaches a sustained level that reflects the greater load (the differences between the two plots is significant, Mann-Whitney test). Both plots were fitted to exponential functions with two sets of variables (French and Torkelli 1994). These curves better described the data than simple exponential functions (Chapman and Smith 1963; Bohnenberger 1981), as has previously been found in restrained preparations (Ridgel et al. 2000). The tonic discharges of a sensillum at different tonic levels from another experiment is plotted in Figure 5E. This animal was exceptional as it held a posture for over 131 seconds through repeated trials of loading and unloading. Despite variability in the response the adapted sensillum discharge is able to reflect the level of force (R squared value = 0.94) if a single posture is held.

Motor responses to changes in body load

Currents applied to the coil also produced activation of motoneurons to specific leg muscles. Figure 6A shows a recording of activities of the trochanteral and tibial extensor muscles while forces were applied using ramp and hold functions (amplitude +/- 3.98 mN) similar to that used in Figure 5. The single trochanteral extensor motoneuron (Ds) responded with a large burst of activity, consisting of an initial phasic discharge followed by tonic activation through the hold phase. In contrast, the tibial extensor was not regularly activated in these tests and, in this sequence, shows only a single spike at the onset of ramp displacement (its activity was apparent when the animal walked or moved). Figure 6B is a plot of the mean firing frequency of the

Figure 6. Motor Responses and Sensory-Motor Integration. A. Responses of motoneurons to two extensor muscles to ramp and hold stimuli applied to the coil. The trochanteral extensor (Ds) fired an intense initial discharge that adapted to a lower, sustained level. The tibial extensor (SETi) only fired a single action potential at the onset of the stimulus. B. Plot of Ds firing frequency at different loading levels for the entire (solid dots) and last 25% of the hold (hollow dots). Both the overall and adapted Ds frequencies reflect the level of load (the mean firing frequency in the interval between these tests was 1.22 spikes/s +/- 0.85 SD). C. Simultaneous recording of the tibial campaniform sensilla (SENSORY nerve 5r8) and the trochanteral extensor (Ds) during application of sine wave current to the coil (Magnet coil) during a single maintained posture. Both the proximal sensillum and the Ds motoneuron fired at their highest frequencies during the negative phase of the current, when the animal was pulled towards the substrate. The distal sensillum fired only during the positive phase. D. Normalized histogram of proximal sensillum and trochanteral extensor activities during 73 cycles of sine wave stimulation. Proximal receptors showed a rapid acceleration of firing during negative current application and reached the maximum frequency near the minimum sinusoidal peak. The trochanteral extensor (Ds) firing was initiated during the same phase and showed its highest frequency following activation of the proximal receptors. E. Reflex excitation of Ds by proximal sensilla - The animal in C. was restrained. Bending forces (Bend Leg) applied to the leg in the direction of forced extension of the femoro-tibial joint excited the proximal sensillum and the Ds motoneuron for the duration of the stimulus. Indentation of the cap (Indent Cap) of a

single proximal receptor produced short latency excitation of the Ds motoneuron. This reflex could contribute to activation of Ds in load tests.


A.
37.5
Troch Ext (Ds)
0
Tibial Ext (SETi)
10
0
Magnet Current
Troch Ext (Ds)
Tibial Ext (SETi)
B.
Ds Firing Frequency (spikes/s)
22
20
18
16
14
12
10
8
6
4
2
3
4
5
Force (mN)
C.
100
Prox Sens
0
50
Troch Ext (Ds)
0
Magnet Current
Sensory N5r8
Troch Ext (Ds)
A: 5.0 mN
C: 4.5 mN
A:0.5 s
C:0.2 s
E: 0.36s bend
0.08 indent
D.
Prox Sens
Ext (Ds)
Current
Prox Sens Freq (spikes/s)
120
100
80
60
40
20
0
Troch Ext (Ds) Freq (spikes/s)
60
50
40
30
20
10
0
Force (mN)
20
10
0
-10
-20
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Phase of Sine Wave
E.
BEND LEG
INDENT CAP
Troch Ext (Ds)
125
0
40
0
Prox Sens
100
0
400
0
Stim
Sensory N5r8
Troch Ext (Ds)

trochanteral extensor at different force levels applied in the same experiment. Both the mean overall discharge and the adapted firing frequencies increase to increments in force level and remain above baseline levels. Thus, activities of the trochanteral extensor can be adjusted to counter variations in the effects of body load upon the leg.

Could the tibial campaniform sensilla contribute to these motor discharges? Figure 6C shows a sequence in which activities of the tibial campaniform sensilla and the trochanteral extensor were recorded simultaneously while sinusoidal current was applied to the coil. In this posture, both the proximal sensillum and the extensor motoneuron (Ds) discharged during the period in which the magnet was being pulled toward the substrate and are inhibited during the opposite phase. While both sensory and motor firing occurred during the same phase, the exact time of onset of firing of the sensillum and the motoneuron varied. Figure 6D is a cumulative histogram constructed from 73 cycles of the sequence shown in 6C. The firing frequencies of the proximal receptor and Ds motoneuron consistently accelerate during the rising phase of the stimulus and the peak of sensory activity precedes the maximum firing of the motoneuron. This pattern is consistent with a known reflex effect of the proximal sensilla upon the Ds motoneuron which is shown in the recording in Figure 6E. This recording was obtained from the same animal as Figure 6C after it was restrained for identification of the sensory units. Mechanical stimulation of the proximal receptor, by bending the leg or indentation of its cuticular cap produced short latency activation of the Ds motoneuron. Thus, the proximal sensillum firing seen during increased load could contribute to motor activation of the trochanteral extensor, if this reflex connection is present in standing animals.

DISCUSSION

These experiments have demonstrated that the tibial campaniform sensilla can show vigorous responses to forces applied to the thorax when the legs are actively used in support and raise the body from the substrate. These discharges appear to be due to mechanical forces acting upon the legs, as application of magnetic fields elicited no sensory or motor responses when the thorax rested against the floor of the arena. In the following we will first review these findings as evidence for the sensitivity of the tibial sensilla to changes in body load, then discuss mechanisms that have been shown to counter load and maintain body height in insects, and finally review the types of afferent information that could contribute to support of body load.

Increasing body load by magnets

When animals stood with the body raised from the substrate, forces applied to the thorax, via magnets attached to the wing base, acted to pull the cockroach toward the floor of the arena and were resisted by the legs. These perturbations did not elicit startle reactions or escape turning and running in any preparations (Nye and Ritzmann 1992). Instead, when the coil was appropriately positioned below the magnet, the animal as a whole simply moved directly down and up, although slight tilting occurred in some trials. Although we were unable to precisely calculate the distance between the thorax and substrate, the changes in position of the magnet or markers on the body that we measured are in a range (0.1 – 0.4 mm) similar to that observed in cockroach running (0.3 mm, Full and Tu 1991, Figure 1). Furthermore, the method of application of the load via magnets attached to the base of the wings provided coupling to the body that was sufficient to produce very short latency sensory discharges to load increases.

Unloading may have been more delayed, as indicated by latency of the movements of the body when the current pushed the magnet away from the substrate. However, the effects of these forces were transmitted to the limbs by the same cuticular, ligamentous and muscular structures by which the animal's weight would be transmitted to the legs. These perturbations also effectively modulated activities of the trochanteral extensor muscle, which has been shown to counter the effects of forces applied to the thorax in other insects (Cruse et al. 1993). These observations support the idea that these stimuli produced forces similar to those occurring when the effect of body weight upon the legs changes.

Campaniform sensilla encode effects of body load upon the legs

The tibial campaniform sensilla showed bursts of activity or modulations of firing frequency at preferred (discrete) phases of the stimulus when repetitive perturbations were applied to the body. Receptors exhibited strict directional sensitivity and the afferent discharge frequency reflected the rate of change of currents applied to the coil (Zill and Moran 1981a; Ridgel et al. 1999, 2000, 2001). Activation of the tibial sensilla to forces derived from the body was also observed in previous experiments that tested responses to lateral displacement of the substrate in freely standing animals (Ridgel et al. 2001). Large platform displacements produced movements of the body and elicited discharges of the receptors that followed the cessation of substrate translation (Ridgel et al. 2001, Fig. 3).

Furthermore, in the present studies, currents applied to the coil that increased load using ramp and hold waveforms were particularly effective stimuli for excitation of the proximal sensilla. Proximal receptors showed phasico-tonic discharges that were elevated for the duration of the stimulus and exhibited hysteresis when force levels declined (Ridgel et al. 2000). This sensitivity to body load could readily contribute to the tonic activities seen in proximal sensilla in freely standing animals (Zill and Moran 1981b). Similar responses have also been observed in mechanoreceptors of crustacea that detect cuticular strain in the legs (Libersat et al. 1987). Those receptors (funnel canal organs) exhibit elevated firing in the stance phase of walking when weights are attached to the thorax. When changes in load were imposed as ramp and hold functions, discharges of the proximal sensilla showed adaptation that had initial very rapid and subsequent slow components. The time course of adaptation could be well described by functions with pairs of exponential variables, as has been utilized to characterize discharges of other arthropod mechanoreceptors (French and Torkelli 1994). However, despite extensive adaptation, the sustained component of proximal sensilla firing could reflect the magnitude of the change in tonic load.

Thus, these results provide direct evidence to support earlier hypotheses that campaniform sensilla of the legs can detect the effects of body load (Cruse et al. 1993). In insects, sensitivities of cuticular mechanoreceptors in the legs to the weight of the body are thought to mediate detection of changes in the orientation of the gravitational vector. These changes can occur when animals traverse irregular terrains or climb vertical or inverted surfaces (Wendler 1966). These signals are thought to aid in adjustments of posture and walking that are made without specialized sense organs for gravity detection, such as vestibular or statocyst systems.

Detecting decreases in load

Discharges of distal tibial campaniform sensilla were often elicited in tests in which load was varied using sine wave functions. Firing of distal receptors occurred during the phase in which the animal was pushed away from the substrate and forces derived from the thorax decreased below baseline levels. Discharges of distal sensilla could also be elicited using half sine waves, which acted to suddenly increase forces and then return them to their original baseline level. Similar responses were obtained from distal receptors in previous studies on restrained preparations when bending forces applied to the tibia were increased and then returned to an offset level (Ridgel et al. 2000). The maximum firing frequencies and numbers of spikes were consistently lower for distal sensilla than was elicited from proximal receptors, consistent with their higher threshold to cuticular strains (Ridgel et al. 2000). These observations support the hypothesis that the distal tibial sensilla can signal force decreases in upright posture in freely standing animals. Sensitivities to decreases in the effect of body load could also contribute to the bursts of distal sensilla that occur at the end of the stance phase of walking (Noah et al. 2001).

Variability in sensory discharges

While the tibial sensilla were able to encode force dynamics and the relative magnitude of changes in load while holding single postures, sensory responsiveness and absolute afferent firing frequencies could readily change if the animal moved its legs to different positions or assumed new postures. Similar variability was also seen in sensory discharges to platform perturbations (Ridgel et al. 2001) in which cockroaches were unrestrained and free to take up a variety of positions and postures. Substantial variations in the magnitude of responses to imposed forces that changed body height have also been reported in stick insects (Kemmerling and Varju 1981; see below). The variability in responsiveness of the tibial campaniform sensilla could be due to a number of factors. The percentage of body load supported by a single leg can vary depending upon the position of a limb relative to the center of mass and the extent of active support by other legs (Full et al. 1991; Pratt 1995). The tibial sensilla also respond to local cuticular strains generated by muscle contractions, which could substantially bias their sensitivities to the effects of body load (Zill and Moran 1981a). These observations support the idea that the discharges of the tibial receptors do not simply reflect the animal's weight, as would a bathroom scale, but instead indicate the extent of support provided by a leg. However, the tibial sensilla can effectively encode parameters of changes in force that can occur at a variety of levels of load while single positions are maintained (Ridgel et al. 2000).

Motor responses to changes in the effects of body load

The trochanteral extensor motoneuron (Ds) was excited when currents were applied to the coil that pulled the animal toward the substrate and increased load. The Ds motoneuron fired bursts during sinusoidal stimulation and discharges were sustained at higher levels when ramp and hold stimuli were applied. These patterns of activation were found to be specific for the trochanteral extensor and not the more distal tibial extensor (SETi). The Ds motoneuron is active during the stance phase of locomotion and its frequency is increased by loading the animal (Pearson 1972). In contrast, the tibial extensor only fires at a low level early in stance but accelerates later in the stance phase (Krauthamer and Fourtner 1978; Larsen et al. 1995; Tryba and Ritzmann 2000).

These findings suggest that the two extensors may be preferentially recruited in countering loads in posture and locomotion: the trochanteral extensor may be preferentially activated to support body weight, while the tibial extensor could be recruited when forces resist forward propulsion, but may be less effective in supporting body load. Further experiments are planned to test these hypotheses.

Parallels with tests of regulation of body height

Motor outputs in response to forces applied to the thorax have also been examined in studies on stick insects that focused upon the regulation of body height (Kemmerling and Varju 1981, 1982; Cruse et al. 1989, 1993). Although specialized sense organs may be present in some systems to prevent contact of the body with the substrate (Eckweiler and Seyfarth 1988; Milde and Seyfarth 1988), the results of studies examining control of body elevation parallel the findings of the present experiments. For example, when forces were imposed upon the thorax that decreased body height, counter forces were developed that opposed the displacement (Kemmerling and Varju 1981). Responses to step displacements had large phase components that occurred at short latencies (5-10 msec) and subsequent tonic components that showed very slow adaptation (Kemmerling and Varju 1982). The magnitudes of the responses were non-linear functions of the amplitude of the imposed vertical displacement when single postures were maintained. However, the amplitude of the compensatory forces showed considerable variability, both between animals and in repetitions of the same stimulus in single animals. Some of this variability was attributed to changes in the 'state of arousal' of the animal (Kemmerling and Varju 1981, p. 130) [consistent with reports that animals can frequently change the gain of feedback from joint angle receptors].

Subsequent experiments by Cruse have shown that activity of the trochanteral depressor muscle is strongly modulated by changes in height imposed during walking movements and that the coxo-trochanteral joint may be a major regulator of body height in stick insects (Cruse et al. 1993). Thus, similar mechanisms may underlie the control systems described for regulation of body height and those that counter the effects of body load.

Changes in load are detected both by sense organs encoding forces and afferents signaling kinematic variables

The present experiments have shown that forces imposed upon the thorax produce changes in the animal's position relative to the substrate and can elicit strong discharges from the tibial campaniform sensilla. These findings suggest that changes in the effects of body load upon the legs most likely are detected both by receptors that monitor kinematic variables such as joint angles and by sense organs detecting forces in the limbs. This hypothesis is supported by a number of previous investigations in invertebrates and vertebrates.

In insects, perturbations that change body load or body height can activate resistance reflexes that are mediated by joint angle receptors (chordotonal organs). For example, locusts that stand on the wall of an arena that is swayed, so that the animal's body is alternately pushed toward and pulled away from the substrate, show regular changes in the angle of the femoro-tibial joint and muscle bursts that are correlated with the phase of the movements (Zill and Frazier 1990; Zill et al. 1992). Motoneuron bursting is also followed by a deceleration in the rate of change of joint angle. In stick insects, increased forces upon the thorax produced tonic changes in body position and elicited sustained forces to counter the displacement (Kemmerling and Varju 1982). The reactions that occur to changes in body height are similar to resistance reflexes elicited by chordotonal organs and have been modeled based upon the torques generated by changes in leg joint angles (Cruse et al. 1992). Furthermore, resistance reflexes can be elicited in freely standing locusts by direct displacement of the ligament of the femoral chordotonal organ, which mimics sudden changes in leg joint angles (Zill 1987; Zill and Jepson-Innes 1988). Prolonged displacements also produced tonic activation of the slow tibial extensor motoneuron that are sustained for the duration of the stimulus, and showed considerable hysteresis upon return to the initial ligament position. In vertebrates, recordings of sense organs in freely standing animals during changes in the effect of body load have shown that bursts of activities can occur in muscle spindles when loading of the thorax is suddenly changed. For example, a light thrust upon the back of a cat that is attempting to stand up from a crouched position, can produce large increases in firing of spindles of leg muscles (Prochazka et al. 1977).

Other studies in vertebrates have shown that both muscle spindles and Golgi tendon organs are probably activated by increased load. For example, activities of leg proprioceptors were recorded from humans that stood freely, but supported themselves by holding a bar (Aniss et al. 1990). Releasing the bar increased loading of the legs and produced firing of tendon organ and spindle afferents, as well as enhanced activities of leg muscles. Sudden increases in load in cats standing on a platform (by lowering the substrate beneath a single leg) produces activation of leg extensors in patterns closely resembling those seen by electrical stimulation of tendon organ afferents in peripheral nerves (Pratt 1995).

These studies suggest that adjustments made to support body load may be based both upon inputs from receptors signaling kinematic variables (muscle length, joint angles) and those that detect forces in the legs. These regulatory processes most probably require the convergence and integration of these inputs in the central nervous system, as has been found in both vertebrates (Jankowska 2001) and invertebrates (Stein and Schmitz 1999). A key advantage of convergence in this process may be the rapidity in which changes are detected by receptors sensitive to force. As is documented in the present study, firing of afferents that detect force dynamics can often occur early in perturbations (Ridgel et al 2001) and precede large changes in joint angles or body position. This rapidity may be utilized by both vertebrates and invertebrates in monitoring the effectiveness of support and adjusting motor outputs to similar advantage.

ACKNOWLEDGMENTS

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CHAPTER 2

FORCE DETECTION IN COCKROACH WALKING RECONSIDERED: DISCHARGES OF PROXIMAL TIBIAL CAMPANIFORM SENSILLA WHEN BODY LOAD IS ALTERED

SUMMARY

We examined the mechanisms underlying force feedback in cockroach walking by recording sensory and motor activities in freely moving animals under varied load conditions. Tibial campaniform sensilla monitor forces in the leg via strains in the exoskeleton. A subgroup (proximal receptors) discharge in the stance phase of walking. This activity has been thought to result from leg loading derived from body mass. We compared sensory activities when animals walked 1) freely in an arena; and 2) upon an oiled glass plate with their body weight supported. The plate was oriented either horizontally (70-75% of body weight supported) or vertically (with the gravitational vector parallel to the substrate). Proximal sensilla discharged following the onset of stance in all load conditions. In addition, activity was decreased in the middle one third of the stance phase when the effect of body weight was reduced. Our results suggest that sensory discharges early in stance result from forces generated by contractions of muscles that press the leg as a lever against the substrate. These forces can unload legs already in stance and assure the smooth transition of support among the limbs. Force feedback later in stance may adjust motor output to changes in leg loading.

INTRODUCTION

Forces that act upon the legs of an animal during standing and walking represent a dynamic equilibrium derived from body loading, inertia and the contraction of limb muscles (Duysens et al. 2000). The detection of these forces is considered an integral component in the control of posture and locomotion (Jacobs and Macpherson 1996). However, few studies have directly recorded the discharges of limb sense organs that monitor forces in freely moving animals (Prochazka 1996; Prochazka and Gorassini 1998; Libersat et al. 1987). It has therefore proven difficult to evaluate the relative contributions of external load and self generated muscle contractions to the information they provide.

We have examined this problem by recording activities of the tibial campaniform sensilla of the cockroach in freely moving animals under conditions in which body loading is altered. Campaniform sensilla encode strains in the exoskeleton (Pringle 1938; Cocatre-Zilgien and Delcomyn 1999). The dendrite of the receptor is linked to an ovoid cap and asymmetrical collar that are embedded in the cuticle (Moran et al. 1971). Forces that are generated by the animal's weight or by the contraction of limb muscles produce strains in the exoskeleton, when leg movements are resisted by contact with the substrate. These strains are distributed through the cuticle in accordance with its thickness and mechanical properties (Zill and Moran 1981; Ramasubramanian 1999). Individual campaniform sensilla are directional and respond to forces that produce compressive strains oriented perpendicular to the cap and collar (Spinola and Chapman 1975). The tibial campaniform sensilla consist of two subgroups, proximal and distal receptors, with mutually perpendicular cap orientations. Previous studies in restrained preparations have shown that the proximal sensilla are activated when forces are applied to the tibia in the direction of extension of the femoro-tibial joint (Zill and Moran 1981a) as should occur in upright posture and locomotion (Ridgel et al. 1999). The phasico-tonic discharge of the sensilla effectively encodes the magnitude and rate of applied forces (Ridgel et al. 2000).

Contractions of leg muscles also produce strain in the exoskeleton when the distal end of the limb contacts a surface, and these strains are known to affect firing of campaniform sensilla (Zill and Moran 1981a; Delcomyn 1991). Contractions of the tibial extensor muscle, for example, produce strains in the tibia (tensions on the dorsal surface) that inhibit activities of the proximal sensilla and excite the distal sensilla (Zill and Moran 1981a). In contrast, the tibial flexor muscle has the opposite effect in exciting the proximal and inhibiting the distal receptors. It is not known if contractions of muscles located more proximally in the leg produce bending forces that affect the receptors, although the tibia is a long cylinder and could readily be strained by their action.

Activation of the campaniform sensilla by bending of the leg or indentation of individual caps elicits reflexes in motoneurons to leg muscles in restrained preparations (Zill et al. 1981). These reflexes affect activities of both the tibial and trochanteral muscles. Stimulation of the proximal sensilla excites the extensors of both joints while activation of the distal sensilla inhibits the extensors and produces excitation of flexor motoneurons. Locally, these reflexes constitute a negative feedback system: the proximal sensilla, for example, excite the tibial extensor and its contraction produces strains that specifically inhibit those receptors. However, the effects of contraction of the trochanteral muscles upon the strains detected by the tibial sensilla are unknown, so it is unclear whether the intersegmental reflexes form a positive or negative feedback system (Ramasubramanian et al. 1999).

The activities of the tibial campaniform sensilla have also been recorded in freely moving animals (Zill and Moran 1981b; Ridgel et al. in press). The receptors are active during the stance phase of walking in a pattern that depends upon the orientation of their cuticular cap: the proximal sensilla initiate firing at the onset of stance, while the distal receptors discharge single or pairs of action potentials immediately prior to leg lifting in swing. Zill and Moran (1981b) considered that the discharges of the proximal sensilla result from application of body weight to the leg, as bursts occur after foot placement. The initial loading of the leg was thought to be resisted by inherent muscle tensions (Hoyle 1978) and by the orientation of the hindleg in stance (Hughes 1952), in which the femoro-tibial joint axis is nearly perpendicular to the substrate. The range of afferent firing frequencies during stance are also similar to those obtained by imposing forces on the leg (bending of the distal tibia) in restrained preparations (Zill and Moran 1981a). The sensory discharges following leg contact could then initiate reflexes to counter the load (Sherrington 1906). This view of sensillum activation has been cited by other authors (Chapman 1998; Burrows 1996), although the original experiments did not examine whether changes in body load altered proximal discharges.

In the present studies, we recorded the activities of the tibial campaniform sensilla in freely moving animals during a variety of loading conditions. These experiments were initiated after preliminary recordings with animals carrying additional weights indicated that the previous interpretation of discharges of the sensilla (that the proximal sensilla encode body load) were insufficient to describe the patterns seen in freely moving animals. Our new studies have provided data to support a reinterpretation of those discharge patterns during walking: the proximal sensilla bursting can be initiated at similar levels even during conditions of substantially reduced load. This finding suggests that their initial discharge reflects forces generated by leg muscles.

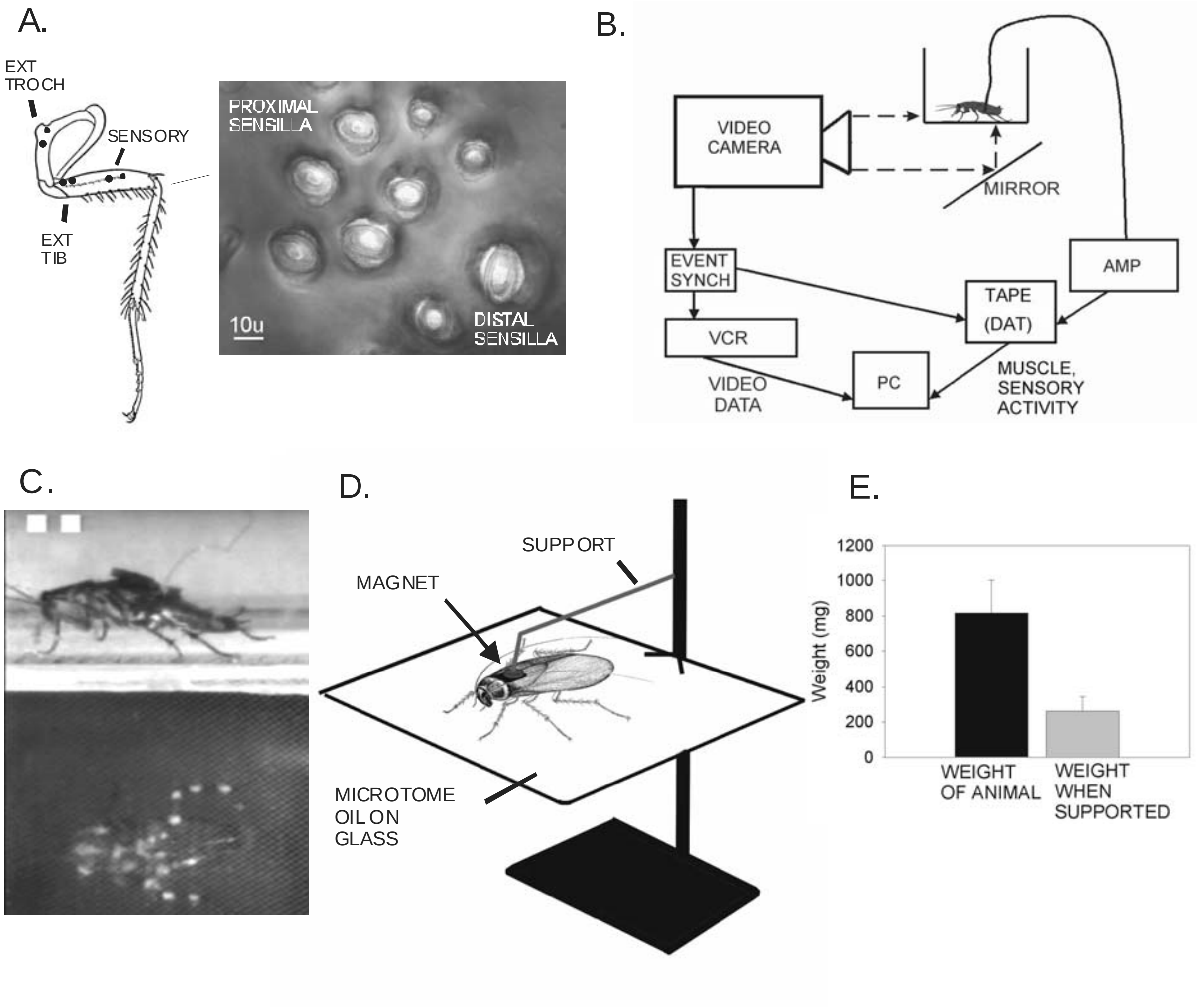
METHODS

Free walking preparations

Animals (n=20) were first restrained on a resin-coated block and pairs of fine wire electrodes were inserted into the left hindleg and positioned to record discharges of the tibial campaniform sensilla (Zill and Moran 1981a; Ridgel et al. 1999, 2000) and the tibial extensor (n=10) or trochanteral extensor (n=10) muscles (Fig. 7A) (Watson and Ritzmann 1998a, b). All wires were tied to the leg and secured with cyanoacrylate adhesive. Small pairs of dots were painted on the coxal, femoral and tibial segments using white nail polish (Wet N Wild) for measurement of leg joint angles (CTr- coxotrochanteral and FT- femoro-tibial joints) in subsequent kinematic analysis. A small harness was then attached to the dorsum of the animal by tying a silk suture through the base of the wings approximately above the middle legs. A piece of tape that was coated with adhesive and steel powder (Steel of West Virginia) was glued to the suture (total weight 40 – 60 mg = 5-7% of body weight) so that it was approximately parallel with the walking substrate.

Preparations were then released into an arena that had plexiglass walls and a floor of fine nylon mesh (Fig. 7B). Walking occurred spontaneously or was elicited at

Figure 7. Experimental setup, sensory and motor unit identification, and controls. A. Left. Sensory and motor activities were recorded in freely moving animals by inserting fine wires into the leg in the coxa (EXT TROCH – Trochanteral extensor muscle) and femur (EXT TIB – Tibial Extensor). Right. Image of wholemount of cuticular caps of the tibial campaniform sensilla. The tibial campaniform sensilla consist of proximal and distal subgroups that differ in cap orientation and responses to cuticular strain. Activities of both subgroups were recorded in the femur (Sensory). B. Experimental setup for data acquisition in freely walking preparations. Animals walked in an arena and were videotaped from the side and from below via a mirror. Muscle and sensory recordings were amplified and stored on tape (DAT). Video and electrical data were synchronized by computer (PC). C. Image of walking animal – Placement and lifting of the feet (tarsi) were clearly visible and used to determine the timing of stance and swing. Leg joint angles were measured from dots painted on legs. D. Walking on Oiled Glass – To study walking with weight reduced, animals were suspended via a harness and magnet above a glass plate made slippery with a thin layer of microtome oil. Video, sensory and motor activities were recorded as in freely walking preparations. E. Weight distribution. Histogram comparing weight of animals when freely standing and when supported by the harness above a laboratory scale. Measured weight was reduced by 75%.


A.
EXT TROCH
SENSORY
EXT TIB
PROXIMAL SENSILLA
10u
DISTAL SENSILLA
B.
VIDEO CAMERA
MIRROR
EVENT SYNCH
AMP
TAPE (DAT)
VCR
VIDEO DATA
PC
MUSCLE, SENSORY ACTIVITY
C.
D.
SUPPORT
MAGNET
MICROTOME OIL ON GLASS
E.
Weight (mg)
1200
1000
800
600
400
200
0
WEIGHT OF ANIMAL
WEIGHT WHEN SUPPORTED

slow to moderate rates by gently stroking the head or abdomen with a small paintbrush. Recordings of sensory and motor activities were stored on multi-channel digital audio tape (TEAC RD-130TE). Walking movements were imaged at 125 or 250 frames per second using a Redlake Motionscope camera (Morgan Hill, CA). Individual walking sequences were downloaded at 30 frames per second from memory to SVHS videotape. The camera was positioned so that it viewed the animal from the side but was slightly angled and looked down on the floor of the cage. The image was of sufficient magnification to visualize the tarsi of the legs (Fig. 7C). While this level of magnification limited the number of steps that could be viewed in a sequence (4-5 maximum), it permitted accurate determination of the time of contact and lifting of the tarsi in the stance phase for subsequent data analysis. A view of the animal from below was also captured in the same frame through a first surface mirror angled at 45 degrees below the arena (see Figs. 7B,C). This view was used for digitization of joint angles and aided in determination of tarsal contact of the contralateral hindleg. Video data were synchronized to the sensory and motor recordings using TTL pulses that produced white squares in the video images. The camera also generated a separate electrical signal at the end of a sequence. Both pulses were stored as channels on the DAT recording of neurophysiological data.

Walking with weight reduced on oiled glass

To study the effects of reduced weight, animals were supported on a rod above a glass plate that was coated with a thin layer of microtome oil (Lipshaw Manufacturing, Detroit) (Fig. 7D; adaptation of method of Tryba and Ritzmann 2000a, b). A magnet attached to the end of the rod exerted sufficient force to hold the animal via the harness and steel-coated tape and prevent him from walking away. After attaching the harness to the magnet, the height of the animal above the substrate was adjusted using micromanipulators that held the support bar and glass plate. Typically, the optimal height was indicated when the animal spontaneously began to walk using all six legs rhythmically in the same cadence. In some experiments, animals were ultimately able to pull the harness from the magnet (if the layer of oil was incomplete or drying). On those occasions, they were observed to continue walking on the substrate using the same patterns of leg movements and could traverse the surface of glass to its edge.

While these preparations were constrained from controlling their own body height above the substrate, the contact between the metal-coated tape and magnet permitted the harness to rotate somewhat. This could allow for small changes in the direction of attempted progression (heading) although continuous turning was not observed. Use of the magnet also permitted the animal to be readily and reversibly removed from the support.

We estimated the extent to which the body weight was supported by positioning the rod above a laboratory scale. Measurements taken when the animals were standing quietly with the legs contacting the scale showed that approximately 70-75 % of the body weight was supported by the rod, in comparison to the same preparations when standing freely (Fig. 7E). In other experiments we also examined sensory and motor activities with the oiled glass plate oriented vertically (see Results). Video images of walking on the glass plate were taken (directly and through mirror) using the same method as in freely moving animals. The timing of tarsal contact and release could be obtained from large numbers of steps in these preparations as the body remained fixed in the field of view.

Controls for sensillum identification

After bouts of walking, animals were restrained and responses to mechanical bending of the tibia were tested. Forces were applied using a probe that was driven by piezo-electric crystal (Zill et al. 1999; Ridgel et al. 1999, 2000). We also stimulated the caps of individual sensilla, producing unitary discharges of amplitude equivalent to those seen during walking. The caps of the sensilla were then ablated and the responses were re-tested. Ablation of all sensilla eliminated the regular discharges seen during stance. In experiments in which the tibial extensor was recorded, we were also able to detect the spikes from the tibial campaniform sensilla on the myographic electrodes, due to their proximity to the dorsal sensory nerve (Zill and Moran 1981a). This allowed us to confirm that units were sensory. Afferent action potentials are conducted from the periphery of the leg towards the central nervous system and thus first appeared on the distal electrodes in the mid-femur and then later in the proximal femur. The sensory action potentials were conducted between the two pairs of recording electrodes with a measurable conduction delay (mean 1.1 msec for proximal sensilla, 0.8 - 0.9 for distal sensilla) that was identical in responses elicited by leg bending and in activities that occurred during walking.

Data analysis

Neurophysiological data were digitally transcribed and analyzed using CED (Spike 2) software. Video sequences were captured to computer with either a Matrox RT2000 or a Pinnacle Systems Miro DC30 plus capture card. Walking sequences were digitized in Motus software (Peak Performance). The times of onset and termination of the stance phases, determined from video images, were entered in a spread sheet. These data were imported into Spike 2 software and synchronized with the transcriptions of neurophysiological recordings based upon the synchronization pulses and the camera off signal. The margin of error of our synchronization was maximally the time between successive video frames (4 msec at 250 frames/sec, 8 msec at 125 frames/sec), as determined by comparison of video frame numbers with the TEAC transcription of pulses.

We utilized the times of tarsal contact and release to construct normalized histograms of sensory and motor activities in the stance phase. The stance phase (time between foot down and up) was divided into 40 bins and sensory and motor frequencies were sampled from spike event channels in software. In some sequences, the femoro-tibial and coxal-trochanteral joint angles were digitized and calculated as two-dimensional segmental angles from the view taken from below the animal. These angles are useful as indicators of joint movement as the hindleg operates in a plane that is nearly parallel with the substrate. We also measured protraction and retraction movements of the prothoracic and mesothoracic legs by digitizing points on the distal end of the femur of each leg. These kinematic data were not used for compilation of stance phase histograms but were only used to document patterns of leg coordination and changes in joint angles during walking (see Results).

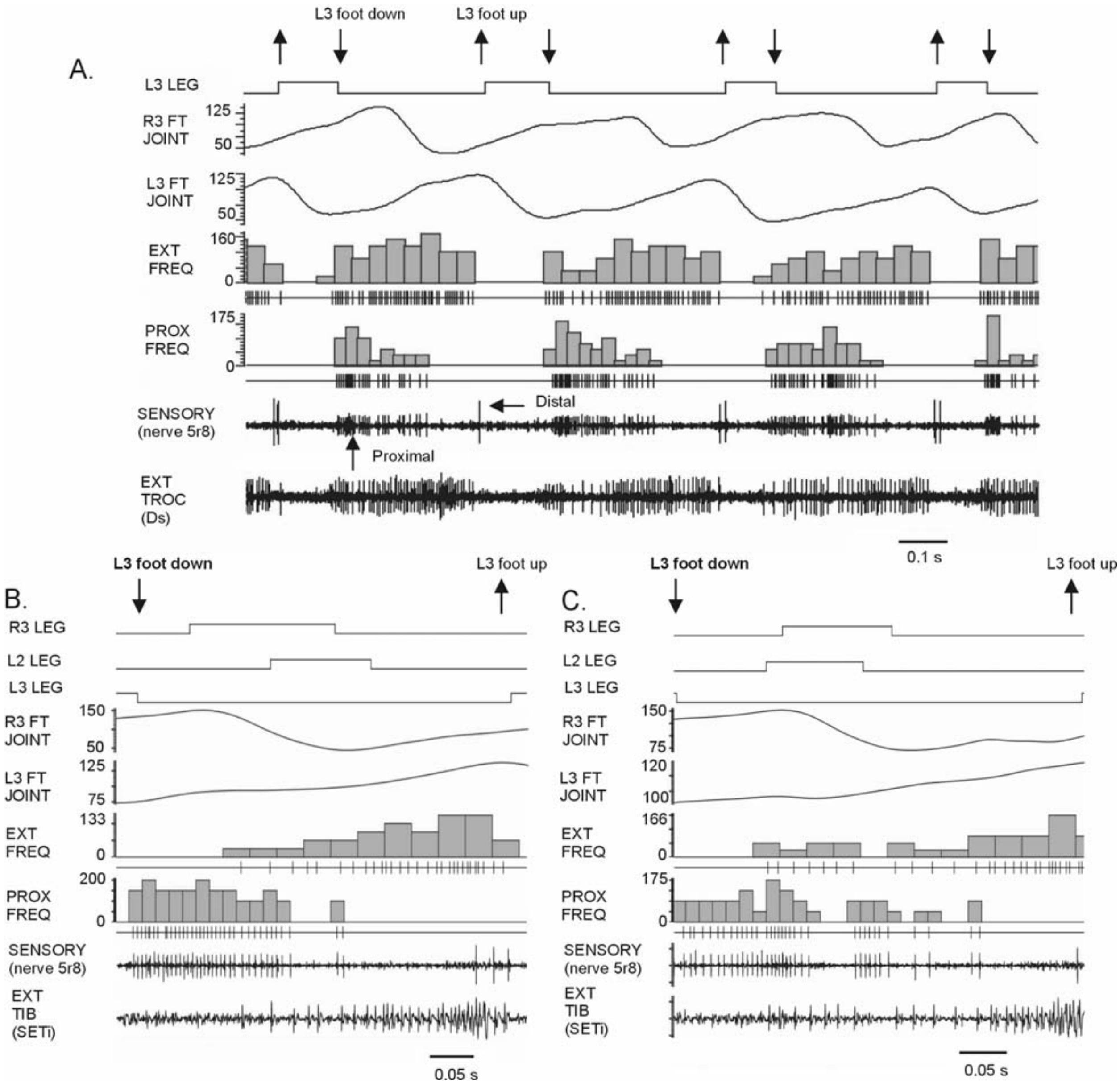
RESULTS

Patterns of activity in freely walking animals

The tibial campaniform sensilla showed a pattern of discharge during the stance phase of walking in freely moving animals of alternating bursts of proximal and distal receptors (Fig. 8). Figure 8A is a recording of activities of the sensilla and the trochanteral extensor in a series of steps, while Figures 8B and 8C show expanded views of stance in single steps in which the tibial extensor was simultaneously recorded. Kinematic data on the time of placement and lifting of the tarsus (feet) of the legs and leg joint angles are indicated in the upper traces, as measured from synchronized video images. The bursts of the proximal receptors were initiated at the time of leg contact in each step (accuracy of video synchronization = +/- 8 msec at 125 frames/second). The mean latency from the onset of foot down to the following proximal sensillum spike was 14.76 msec +/- 17.2 SD (n= 71 steps from 7 animals), or within 1-2 video frames. Activities of the sensilla continued at lower rates later in stance but could show secondary accelerations in firing or separate bursts. In some steps, these increases were correlated with lifting of the contralateral metathoracic or ipsilateral mesothoracic legs (Fig. 8C) while in other steps this correlation was less apparent (Fig. 8B). Activities of the proximal receptors generally ceased during the last part of the stance phase.

Discharges of the proximal sensilla were also correlated with activities of the leg extensor muscles. At slow walking rates, the trochanteral extensor firing was initiated immediately prior to the onset of stance. Trochanteral extensor bursts continued through the middle part of the stance phase and often declined prior to swing. Firing of the proximal receptors regularly followed the onset of bursting of the trochanteral

Figure 8. Activities during Free Walking. A. Recordings of activities of the tibial campaniform sensilla (SENSORY nerve 5r8) and trochanteral extensor muscle (EXT TROCH (Ds)) were synchronized with kinematic data. The femoro-tibial (FT) joint angles of both hindlegs (L3 FT JOINT, R3 FT JOINT) and time of foot contact and lift (L3 LEG) were measured from video images. Proximal sensilla (smaller spikes in SENSORY trace) fire following leg contact and discharges continue through the first half of stance (PROX FREQ). Onset of firing of the slow trochanteral extensor precedes foot placement and the proximal discharge. The L3 FT joint extends during stance and is in antiphase with the FT joint angle of the opposite hindleg. B. and C. Recordings of activities of tibial sensilla, tibial extensor muscle and timing of lifting of adjacent legs during a single stance phase of leg L3. Proximal sensilla firing after leg contact continues through time of lifting of the opposite hindleg (LEG R3) and adjacent middle legs (LEG L2). Afferent firing increases during swing of other legs in some steps (C) but not others (B). The slow extensor firing frequency accelerates through the stance phase.


A.
L3 foot down
L3 foot up
L3 LEG
R3 FT JOINT
125
50
L3 FT JOINT
125
50
EXT FREQ
160
0
PROX FREQ
175
0
SENSORY (nerve 5r8)
Distal
Proximal
EXT TROC (Ds)
0.1 s
B.
L3 foot down
L3 foot up
R3 LEG
L2 LEG
L3 LEG
R3 FT JOINT
150
50
L3 FT JOINT
125
75
EXT FREQ
133
0
PROX FREQ
200
0
SENSORY (nerve 5r8)
EXT TIB (SETi)
0.05 s
C.
L3 foot down
L3 foot up
R3 LEG
L2 LEG
L3 LEG
R3 FT JOINT
150
75
L3 FT JOINT
120
100
EXT FREQ
166
0
PROX FREQ
175
0
SENSORY (nerve 5r8)
EXT TIB (SETi)
0.05 s

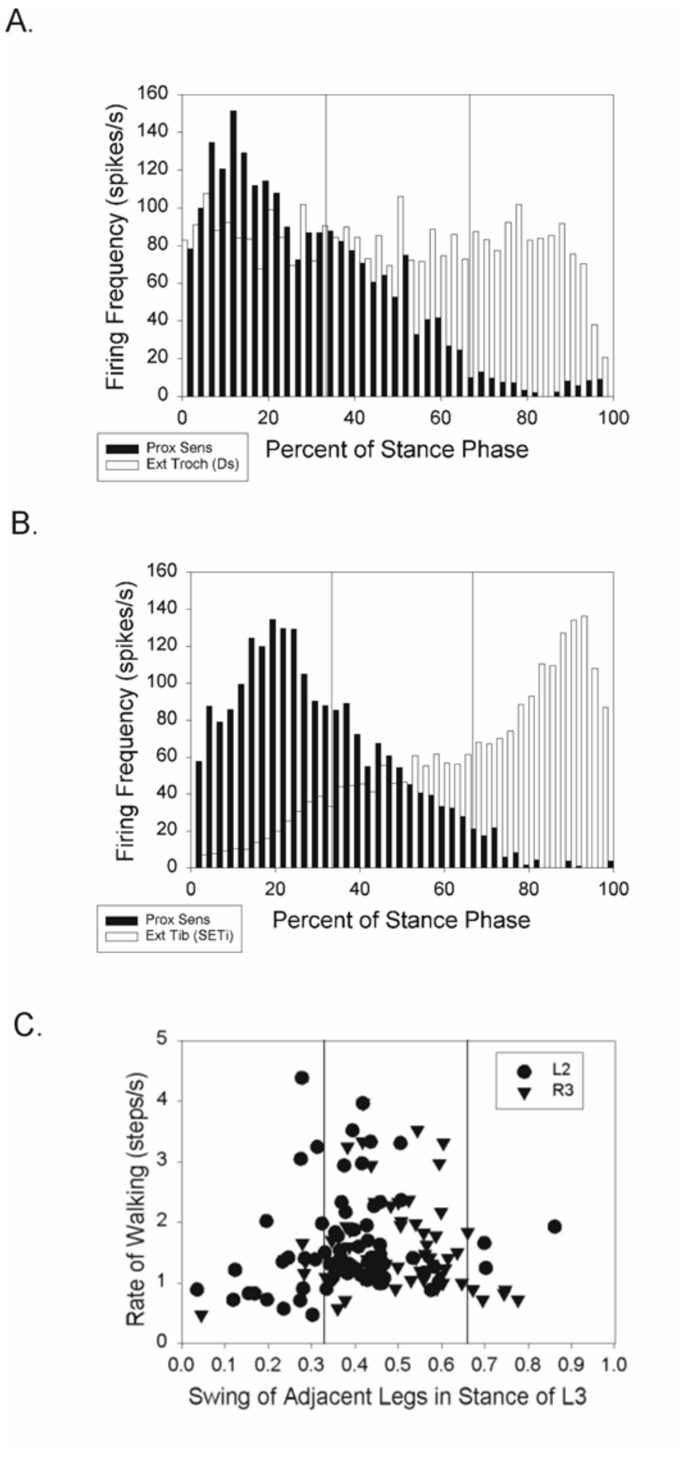
extensor motoneuron (Ds) but subsequent sensory and motor activities were not directly correlated in firing frequency. In contrast, the tibial extensor (SETi) typically only became active later in stance. Firing was initiated at a low level but accelerated within a burst. The femoro-tibial joint also extended during this period at a relatively constant rate (Watson and Ritzmann 1998a). The proximal sensillum firing was reciprocally related to the frequency of discharge of the tibial extensor motoneuron (SETi). The tibial extensor firing reached a peak during the last third of stance, often showing a sharp acceleration that preceded leg lifting in swing (Tryba and Ritzmann 2000a, b).

Firing of the distal sensilla was generally limited to the end of the stance phase in short bursts that typically occurred immediately prior to leg lifting in swing.

Analysis of sensory and motor activities: subdivisions of the stance phase

In order to quantify sensory and motor activities, we constructed normalized histograms of discharges in the stance phase. Steps were analyzed from bouts of walking at slow-moderate rates (less than 5 steps/second), as activities of sensory units were more difficult to discern in more rapid walking. The onset and termination of stance were determined from the video data that was acquired simultaneously and this interval was divided into 40 bins. Figure 9A is a cumulative histogram of firing of proximal sensilla and the slow trochanteral extensor motoneuron (Ds) from 28 steps taken by 2 animals. The mean rate of walking was 1.4 steps/sec and the mean firing frequencies were 57.0 spikes/second for the proximal receptor and 81.6 spikes/second for the Ds motoneuron. Figure 9B is a similar histogram of firing of proximal sensilla and the slow tibial extensor motoneuron from 49 steps taken by 5 animals. The mean walking rate in this group was 1.3 steps/sec +/- 0.4 SD (range .55-2.7 steps/second). The mean firing

Figure 9. Leg Activities During Free Walking. A and B. Normalized histograms of activities of proximal sensilla (black bars), the trochanteral extensor (white bars in A) and tibial extensor motor neurons (white bars in B) during the stance phase. The proximal sensilla firing is initiated at the onset of stance. The discharge increases and then declines later in the stance phase. The trochanteral extensor motor neuron (Ext Troch (Ds)) is active at a relatively constant frequency throughout the stance phase and declines prior to leg lifting. The tibial extensor motor neuron (Ext Tib (SETi)) shows little activity at the onset of stance but its frequency increases to a maximum just prior to the lifting of the leg into the swing phase. Tibial extensor firing is reciprocal with activity of the sensilla. C. Plot showing midpoints of swing phases of legs L2 and R3 with respect to the normalized stance phase of L3. At most walking rates, the time of swing of the adjacent legs occurred in the second third of stance of L3.


A.
Firing Frequency (spikes/s)
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Percent of Stance Phase
Prox Sens
Ext Troch (Ds)
B.
Firing Frequency (spikes/s)
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Percent of Stance Phase
Prox Sens
Ext Tib (SETi)
C.
Rate of Walking (steps/s)
5
4
3
2
1
0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Swing of Adjacent Legs in Stance of L3
L2
R3

frequencies during the entire stance phase was 53.1 spikes/second for the proximal sensillum and 56.4 spikes/second for the tibial extensor motoneuron.

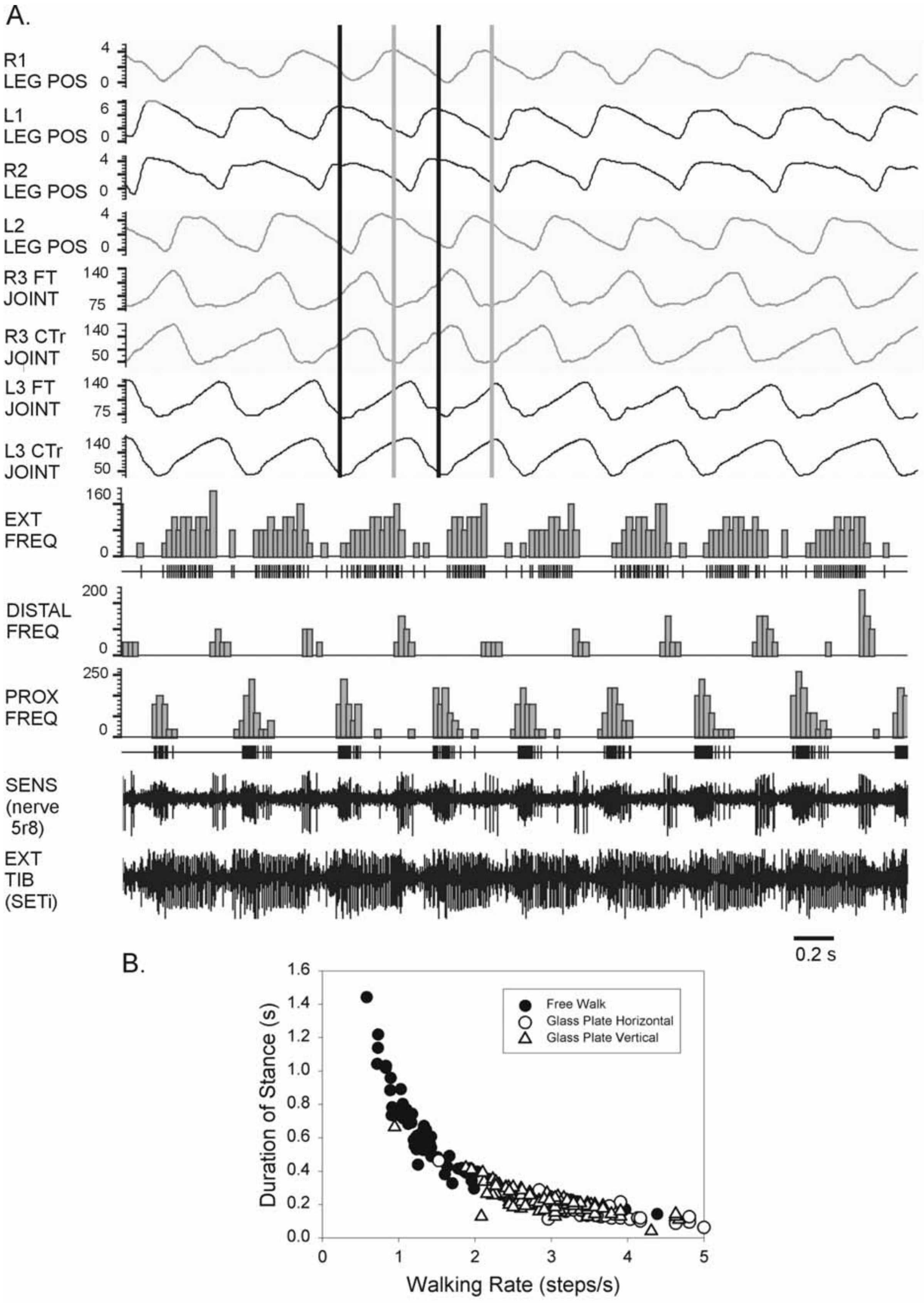
Both histograms demonstrate that, on average, the maximum firing of the proximal sensilla did not occur upon leg contact but reached a peak within the first 15-25 percent of stance. The discharge declined later in the stance phase, but could show additional small increases or accelerations. Proximal sensilla firing generally ceased at or near the start of the last one third of stance (see below). These plots also demonstrate the differing relationship of afferent firing with the two extensors of the leg: proximal discharges are concurrent with firing of the trochanteral extensor early in stance but the afferent frequency is reciprocal with that of the tibial extensor later in the stance phase.

We also quantified kinematic data on the patterns of movements of other legs to establish a framework for subsequent analysis. Lifting of those legs in swing should increase load in the legs in stance and could result in transient increases in afferent firing. This effect would be reduced if the body weight was supported. Examination of the timing of movements of the adjacent (L2 - left mesothoracic) and contralateral (R3-right metathoracic) legs showed that these legs entered into swing after the onset of sensillum firing in leg L3. We quantified these data by calculating the time of the midpoint of swing of the L2 and R3 legs in the cycles of leg L3 (Fig. 9C). Although the timing of movements depended upon the particular pattern of leg coordination, the midpoint of the swing phase of leg L2 was 0.40 +/- 0.16 and leg R3 was 0.47 +/- 0.28 in the stance phase of leg L3 (86 steps from 6 animals). We therefore divided the stance phase of the hindleg (L3) into thirds (0-33%, 33-67% and 67-100% time) for the purpose of description and analysis. In slow walking, these intervals approximately represent 1) first period - adjacent legs on substrate, 2) second period - swing of adjacent legs, 3) third period - adjacent legs again on substrate.

Activities when suspended above a slippery surface

Animals readily showed walking movements when supported above a glass plate that was covered with a thin layer of microtome oil. When the body height was appropriately adjusted, these preparations could show prolonged bouts of activity that closely resembled walking by intact animals, including the utilization of tripod gaits. Figure 10A shows part of an episode in which activities of the tibial campaniform sensilla and tibial extensor muscle were recorded and synchronized with kinematic measurements of leg movements. The entire bout consisted of 50 consecutive steps taken over 28 seconds at a mean rate of 1.76 steps/second. The movements of the front and middle legs were measured in these traces as protraction/retractions along the body axis (Fig. 10 upper traces), while the angles of the femoro-tibial and coxo-trochanteral joints of both hindlegs were digitized (Fig. 10 middle traces). Both joints of the hindlegs were flexed during the swing phase and extended during stance, as is seen in walking of freely moving preparations (Watson and Ritzmann 1998a; Tryba and Ritzmann 2000a). Movements in the two hindlegs were also in antiphase, as is characteristic of walking, and were coordinated in a tripod pattern with the protraction and retractions of the anterior pairs of legs. Thus, the coordination of leg movements in these animals could resemble those seen in walking, despite the fact that the effects of body weight upon the legs were substantially reduced (see Epstein and Graham 1983 for similar results in stick insects). A major difference in these episodes versus walking

Figure 10. Walking on Oiled Glass with Weight Supported A . Measurements of joint angles of the hindlegs and protraction/retraction movements of the middle and front legs were synchronized with recordings of sensory and motor activities (joint angles: CTr – coxotrochanteral, FT – femoro-tibial joints of L3 and R3; protract/retract: leg pos of R2, L2, R1, L1). Leg movements and changes in joint angles occurred in patterns similar to walking of freely moving animals and approximated a tripod gait in this sequence. (Grey and dark vertical lines indicate the onset of stance in legs R3 and L3). Motor (EXT TIB SETi)) and sensory (SENS (nerve 5r8)) activities also resembled patterns seen in animals walking in the arena. Proximal sensilla fired at the onset of joint extension (L3CTr JOINT, L3FT JOINT) but bursts were abbreviated in duration (PROX FREQ). Distal sensilla fired prior to leg lifting in swing (DISTAL FREQ). B. Plot of durations of stance phase vs. rate of walking. The duration of stance decreased at faster rates in both free moving and supported preparations. Animals walked faster over the slippery surface but data from both preparations form an overlapping continuum.

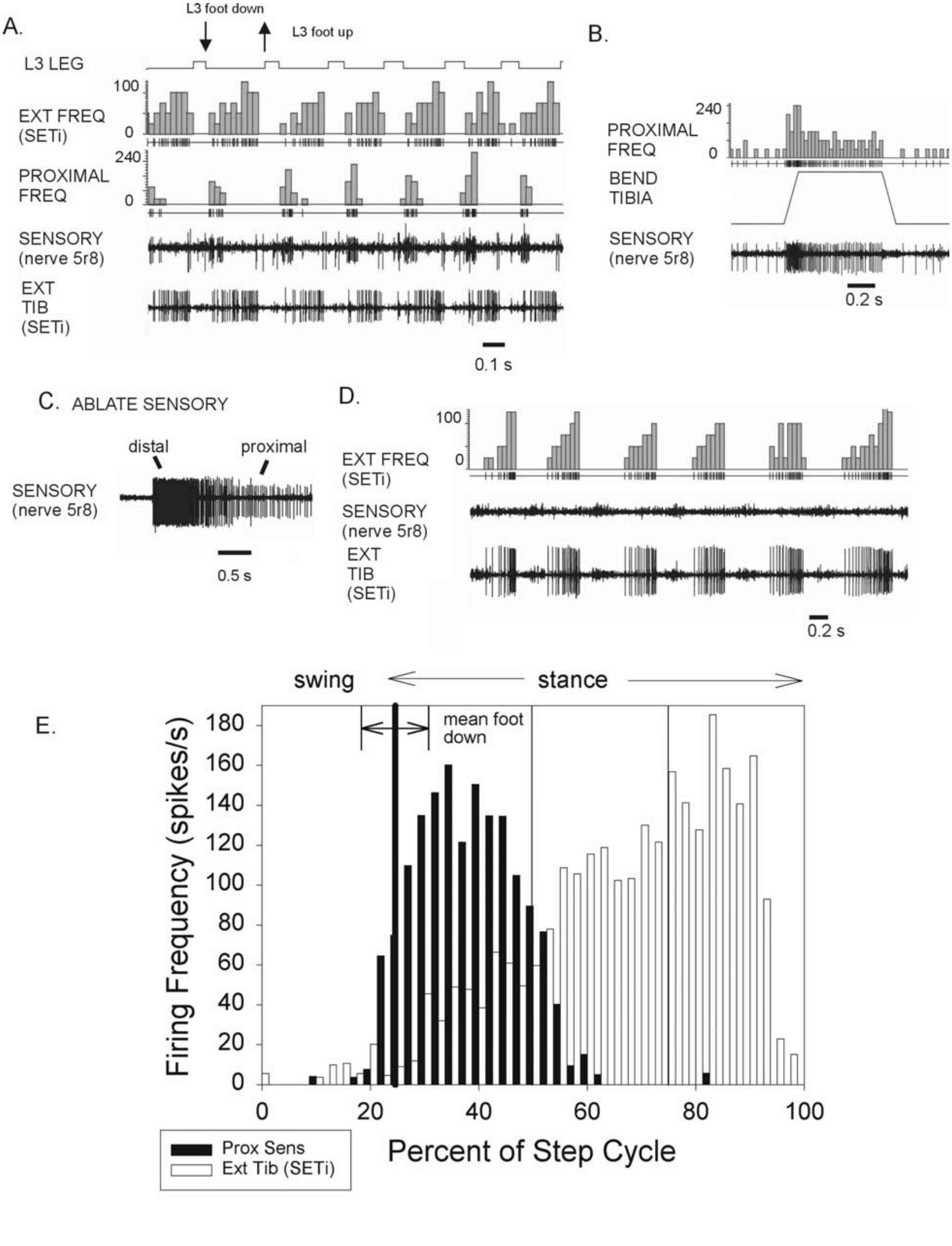

A.
R1 LEG POS
4
0
L1 LEG POS
6
0
R2 LEG POS
4
0
L2 LEG POS
4
0
R3 FT JOINT
140
75
R3 CTr JOINT
140
50
L3 FT JOINT
140
75
L3 CTr JOINT
140
50
EXT FREQ
160
0
DISTAL FREQ
200
0
PROX FREQ
250
0
SENS (nerve 5r8)
EXT TIB (SETi)
0.2 s
B.
Duration of Stance (s)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
Walking Rate (steps/s)
Free Walk
Glass Plate Horizontal
Glass Plate Vertical

of freely moving animals was their duration, which could last for minutes. The constancy of stepping frequency was rarely seen in walking in the arena. Animals also tended to walk more rapidly. Fig. 10B is a plot of the durations of the stance phase at different rates of stepping during walking in the arena and upon the glass plate (total 259 steps; 78 free walk, 73 horizontal glass, 108 vertical glass). While the rate of walking on the slippery surface was faster, the data form a continuous curve with points that clearly overlap from the two data sets. We should also note that these rates were in the range of slow walking for cockroaches, rather than running or locomotion following escape turning.

Recordings of sensory and motor activities in these preparations showed patterns that were also similar to those seen in freely moving animals (Figs. 10, 11A). The tibial receptors discharged in alternating bursts in which proximal sensilla firing followed leg contact while distal receptors were activated prior to lifting of the leg in swing. We confirmed that these afferent recordings were derived from the tibial sensilla by restraining animals and characterizing responses to bending forces applied to the tibia (Fig. 11B). We also ablated the caps of the sensilla (Fig. 11C) which produced discharges of similar amplitudes to those seen in walking. When animals were subsequently retested on the oiled glass, these ablations eliminated bursting in the sensory recordings (Fig. 11D). Activities in leg muscles persisted after elimination of the tibial sensilla, although we did not systematically study the effects of sensory ablations on motor outputs or leg movements.

The characteristics of firing of the tibial sensilla differed in walking with weight supported. Figure 11E is a normalized histogram of sensory and motor activities during

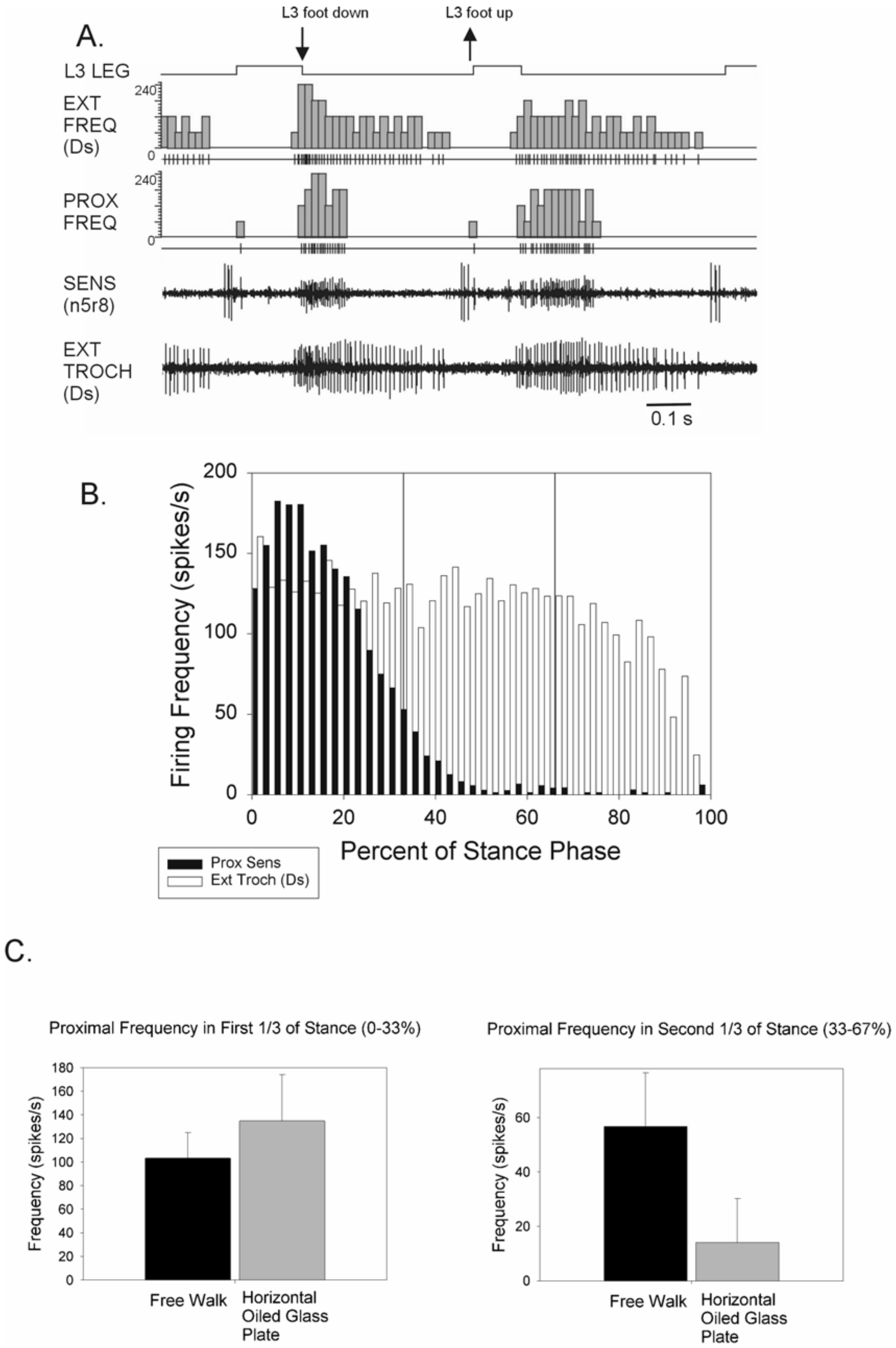
Figure 11. Identification of Sensory Units and Normalized Histogram of Activity During Walking on Oiled Glass. A-D. Ablation of tibial campaniform sensilla eliminated afferent discharges. Sensory recordings showed alternating bursting of small and large units during walking on the glass (A). Preparations were then restrained and bending forces were applied to the tibia (B). Bending toward forced extension produced activation of a proximal sensillum of amplitude equivalent to the small unit seen during walking. Ablation of all the tibial sensilla (C) produced injury discharges from both large and small units. Sensillum ablation eliminated all sensory bursting when animals were retested on the glass (D). E. Histogram of proximal sensillum and tibial extensor activities during the step cycle, normalized from the start of the swing phase. Proximal receptors fired following the onset of stance (vertical bar and arrows = mean foot down +/- SD) but abruptly decreased by mid stance. The tibial extensor (Ext Tib (SETi)) showed a normal pattern of acceleration during the stance phase.


A.
L3 foot down
L3 foot up
L3 LEG
100
EXT FREQ
(SETi)
0
240
PROXIMAL
FREQ
0
SENSORY
(nerve 5r8)
EXT
TIB
(SETi)
0.1 s
B.
240
PROXIMAL
FREQ
0
BEND
TIBIA
SENSORY
(nerve 5r8)
0.2 s
C. ABLATE SENSORY
distal
proximal
SENSORY
(nerve 5r8)
0.5 s
D.
100
EXT FREQ
(SETi)
0
SENSORY
(nerve 5r8)
EXT
TIB
(SETi)
0.2 s
E.
swing
stance
mean foot
down
Firing Frequency (spikes/s)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Percent of Step Cycle
Prox Sens
Ext Tib (SETi)

walking on the oiled glass for 36 steps of a single animal. Proximal activity consisted only of a short, high frequency burst following leg contact that rapidly declined. The activity of the proximal sensillum was higher during the initial third of stance than that seen in free walking animals. The firing of the proximal receptors showed a pronounced reciprocity of activity with the tibial extensor that was similar to that seen in free walking. The slow extensor again showed the pattern of acceleration of activity within a burst that was seen in freely moving animals (Fig. 8A).

Figure 12A shows a recording of activities of the trochanteral extensor motoneuron (Ds) and tibial campaniform sensilla in walking upon an oiled glass plate from the same preparation that was shown freely walking in Figure 8A. The slow trochanteral extensor became active prior to placement of the tarsus on the glass substrate. Activation of the proximal sensillum occurred at foot contact following the onset of extensor firing. The mean latency from the time of foot down to the following proximal spike was 6.0 ms +/- 7.2 SD (100 steps). Figure 12B is a normalized histogram that plots the firing of the trochanteral extensor motoneuron (Ds) and the proximal receptors during the stance phase (determined by tarsus down/up in videos) for 82 steps taken by 2 preparations. The mean rate of walking was 2.9 +/- 0.9 steps/sec and the mean firing frequencies were 49.0 spikes/second for the proximal receptor and 116.5 spikes/second for the Ds motoneuron. Both the motoneuron and the sensory receptors are active at the start of stance. The sensillum firing is largely restricted to the first one third of the stance phase and declines rapidly, while the trochanteral extensor burst continues and declines only during the last one third of stance.

Figure 12. Sensory and Trochanteral Extensor Activities in Walking When Supported A. Recordings showing activities of proximal sensilla (SENS (n5r8), PROX FREQ)) and the trochanteral extensor (EXT TROCH (Ds)) during walking on oiled glass. Ds is activated prior to foot contact and bursting of the proximal receptor and continues to fire throughout the stance phase. B. Normalized histogram (similar to Fig. 3) showing activities of proximal sensilla and Ds during stance. The proximal receptors are active at a high frequency following the onset of stance but the response rapidly declines by the second third of stance. C. Comparison of firing frequencies of proximal sensilla during the first and second thirds of the stance phase in walking in the arena vs. supported above the glass plate. During the first third of stance, sensilla fire at a significantly higher rate in walking on oiled glass. In the second third, the firing frequency is lower when supported.


A.
L3 foot down
L3 foot up
L3 LEG
EXT FREQ (Ds)
240
0
PROX FREQ
240
0
SENS (n5r8)
EXT TROCH (Ds)
0.1 s
B.
Firing Frequency (spikes/s)
200
150
100
50
0
0
20
40
60
80
100
Percent of Stance Phase
Prox Sens
Ext Troch (Ds)
C.
Proximal Frequency in First 1/3 of Stance (0-33%)
Frequency (spikes/s)
180
160
140
120
100
80
60
40
20
0
Free Walk
Horizontal Oiled Glass Plate
Proximal Frequency in Second 1/3 of Stance (33-67%)
Frequency (spikes/s)
60
40
20
0
Free Walk
Horizontal Oiled Glass Plate

In order to quantitatively evaluate proximal sensillum firing in free walking vs. weight-supported animals, we utilized the pooled data in Figures 9A, 9B and 12B and compared the discharges during the first one third and second one third of the stance phase. These data are shown as histograms in Figure 12C. The mean discharge rate of the proximal sensillum during the first one third is higher in walking on the oiled glass than in free walking. Afferent firing is lower during the second one third of stance with weight supported (Student's t-test p= 0.05 first third, p = 0.05 second third). Thus the discharges of the proximal sensilla persist, and are somewhat enhanced, in the first one third of stance with the body weight supported. However, the subsequent discharge in the middle third of stance is reduced when walking on a slippery surface. This reduction could result from the decrease in load bourn by the legs but could also potentially be effected by the increase in rate of walking seen in animals on the oiled glass. The slightly faster walking speed of animals on the greased plate prevented an extensive comparison of steps taken at the same walking rates. However, analysis of the stance phase activities of steps (43 steps total, 10 steps free walking, 33 oiled glass) taken in the range of walking speeds that were overlapping (1.5-2.9 steps/second) still showed a significant decrease in proximal firing during the middle one third of stance in supported vs. free walking animals (free walk proximal frequency = 41.6+/-21.0; supported discharge frequency = 20.9 +/- 22.9; Student's t-test p = 0.05).

Activities when walking on a vertical slippery surface

The finding that proximal bursting persisted in animals that walked with the weight of the thorax supported implied that the early component of the discharge did not result from leg loading from the body. However, the weight of the legs was still supported by the substrate and could have contributed to the afferent discharge. To eliminate the effects of the leg mass, we suspended animals (n=7) against the plate while it was oriented vertically (Fig. 13A). In these circumstances, the gravitational vector would act to pull the leg toward the caudal end of the animal and not down upon the substrate, so the effect of the weight of the distal leg was further minimized.

Animals readily attempted to walk on the vertically oriented glass using patterns of leg movements that could resemble those seen in free walking or when the plate was horizontal (Fig. 13B) (Duch and Pflüger 1995). During 'vertical walking' sequences the CTr and FT joints were flexed during the phase in which the leg was in swing and were extended during stance and legs could be utilized in tripod gaits, as is characteristic of walking but not searching movements (Tryba and Ritzmann 2000 a,b). During bouts of searching in the present experiments, the hindlegs were most often extended during swing and flexed during stance, as has been previously reported (Tryba and Ritzmann 2000a).

Motor activities in these preparations also generally resembled walking when the glass was horizontal. The SETi showed an acceleration in discharges during stance (Fig. 13C) while the Ds motoneuron could fire prior to leg placement (Fig. 13D), although the specific times and patterns of activation of the extensors varied more than that seen in horizontal or free walking. Some myographic recordings showed activities close to the time of foot placement, during vertically oriented walking, that apparently resulted from cross talk from flexor muscles (Fig. 13D). Increases in flexor activity might be expected to occur during the swing phase as protraction of the leg required lifting its mass vertically against the gravitational vector.

Figure 13. Activities in Walking on Vertical Glass - A. Setup. The glass plate was placed in a vertical position in order to change the effects of gravity on the animal. B. Measurements of joint angles of the hindlegs and protraction/retraction movements of the middle and front legs synchronized with recordings of sensory and motor activities as in Figure 4 (joint angles: CTr – coxotrochanteral, FT – femoro-tibial joints of L3 and R3; protract/retract: leg pos of R2, L2, R1, L1). Leg movements occurred in patterns similar to walking of freely moving animals and could be coordinated in tripod gaits, as in this sequence. Motor (EXT TIB (SETi)) and sensory (SENS (nerve 5r8)) activities in walking on vertically oriented glass resembled patterns seen when the surface was horizontal. Proximal sensilla fired at the onset of joint extension (L3 CTr JOINT, L3 FT JOINT) in short bursts (PROX FREQ). C. Expanded section of B. Proximal firing was intense following foot contact and ceased after onset of tibial extensor activity. D. Recording of sensory and trochanteral extensor activity – Ds motoneuron firing was similar to that seen in walking on horizontal glass. E. Normalized histogram showing pattern of firing of Ds and the proximal sensilla in walking on vertical oiled glass. The proximal receptors are active at a high frequency following the onset of stance even though the effects of body load are eliminated. F. Proximal frequency in first third and second third of stance in walking in the arena vs. on a vertically oriented substrate - Sensilla fire at a significantly higher rate during the first one third of stance while on vertical glass, while firing is decreased during the second one third.

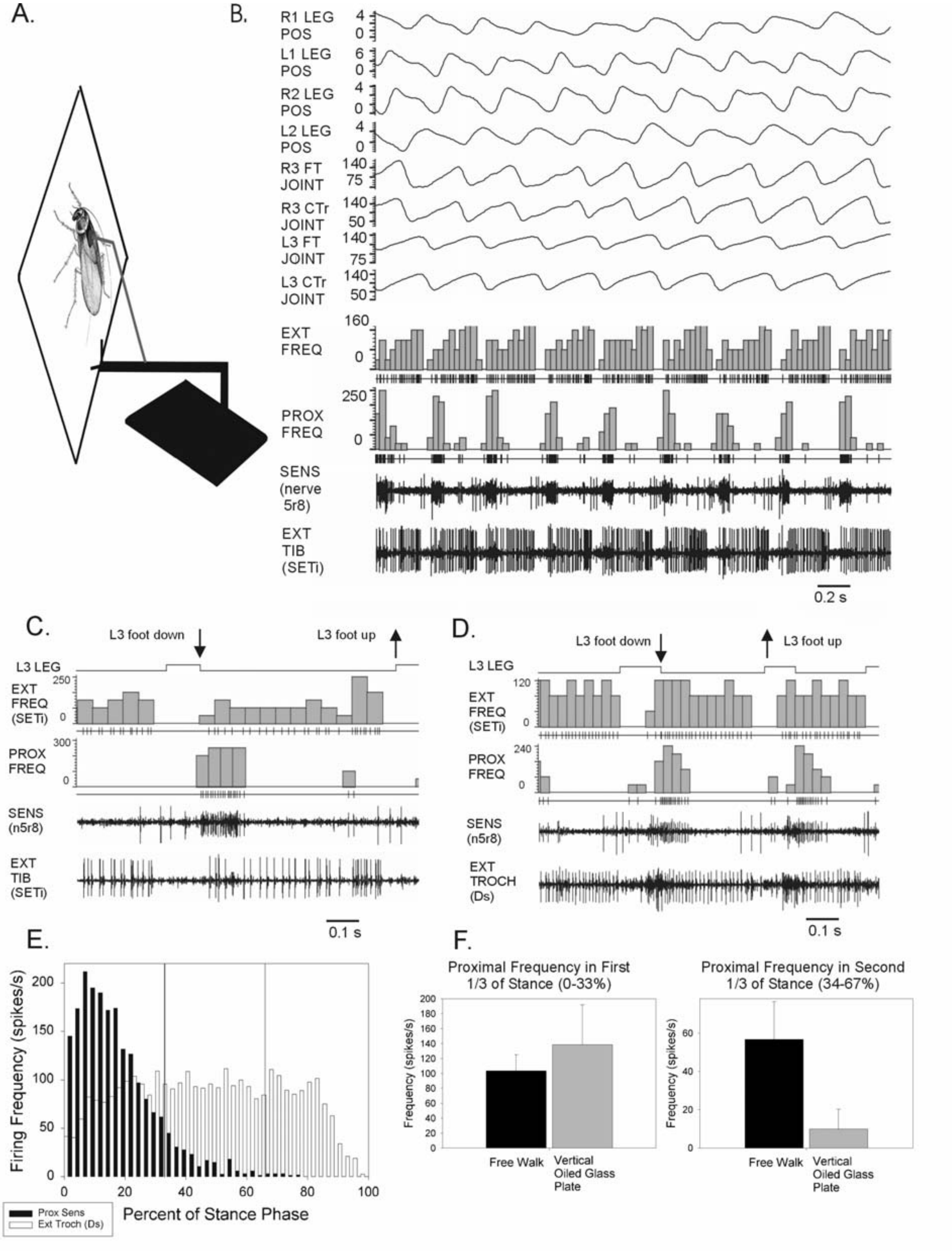

A.
B.
R1 LEG POS
4
0
L1 LEG POS
6
0
R2 LEG POS
4
0
L2 LEG POS
4
0
R3 FT JOINT
140
75
R3 CTr JOINT
140
50
L3 FT JOINT
140
75
L3 CTr JOINT
140
50
EXT FREQ
160
0
PROX FREQ
250
0
SENS (nerve 5r8)
EXT TIB (SETi)
0.2 s
C.
L3 foot down
L3 foot up
L3 LEG
EXT FREQ (SETi)
250
0
PROX FREQ
300
0
SENS (n5r8)
EXT TIB (SETi)
0.1 s
D.
L3 foot down
L3 foot up
L3 LEG
EXT FREQ (SETi)
120
0
PROX FREQ
240
0
SENS (n5r8)
EXT TROCH (Ds)
0.1 s
E.
Firing Frequency (spikes/s)
0
50
100
150
200
0
20
40
60
80
100
Percent of Stance Phase
Prox Sens
Ext Troch (Ds)
F.
Proximal Frequency in First 1/3 of Stance (0-33%)
Frequency (spikes/s)
0
20
40
60
80
100
120
140
160
180
200
Free Walk
Vertical Oiled Glass Plate
Proximal Frequency in Second 1/3 of Stance (34-67%)
Frequency (spikes/s)
0
20
40
60
Free Walk
Vertical Oiled Glass Plate

Figure 13E is a normalized histogram, similar to Fig. 12B, showing the activities of proximal sensilla and the trochanteral extensor motoneuron during the stance phase of walking on the vertical glass surface (71 steps taken by 1 animal). As was consistently seen in all preparations, the patterns of sensory and muscle activities are similar in walking on both the vertical and horizontal surfaces. Proximal activity reaches a maximum shortly after the onset of stance, but the discharge is restricted mostly to the first one third of the stance phase and declines rapidly. The trochanteral extensor is active throughout the stance phase and shows a decline in firing frequency only immediately prior to swing. Figure 13F compares the mean discharges of proximal sensilla during the first one third and second one third of the stance phase in preparations on the vertical glass and in freely moving animals. The discharge rate of the proximal sensillum during the first one third is higher in walking on the oiled glass than in free walking. Afferent firing is also lower during the second one third of stance. These differences are significant for both the first and the second one third (Paired t tests, both tests $p<0.01$). Thus the patterns of sensory activities are similar in both preparations when the effect of weight is reduced, even though the orientation of the gravitational vector is changed.

DISCUSSION

Sensory discharges early in stance persist when body load is reduced

The present study has shown that the proximal tibial campaniform sensilla discharge following leg contact during the stance phase of walking under a variety of loading conditions. The initial component of the discharge, attained during the first 33% of stance, reached firing frequencies that exceeded 100 spikes/second both in animals walking freely in the arena and in conditions in which body load was substantially supported. Similar discharges occurred when the substrate was oriented vertically and the gravitational vector was parallel to the walking surface. These findings are apparently inconsistent with the interpretation that this discharge results from leg loading in support of body weight (Zill and Moran 1981b; see also Chapman 1998, p. 161 and Burrows 1996 p. 397 after Zill and Moran). Furthermore, afferent firing of campaniform sensilla does not appear to result merely from leg contact with the substrate, as occurs in discharges of tactile hairs or receptors of the tarsus (foot) (Newland 1991). The tibial sensilla are located in the proximal part of the tibial segment, some distance from the substrate, and are activated by strains that result from forces transmitted through the exoskeleton. Analysis of synchronized kinematic and neurophysiological data (see Methods) also indicates that the peak discharge of the receptors is often delayed after foot placement, suggesting that afferents are not merely detecting other parameters related to the surface or vibrations transmitted through the substrate. Instead, these findings support the idea, discussed below, that the sensillum firing is the result of forces generated by the animal's muscles following foot placement. In the following we will first review the behavior of animals walking with their weight supported then discuss the sources of forces that could contribute to the sensory discharges we observed. We will also review our findings in the context of the functions of force feedback during walking.

Behavior of animals upon the oiled glass with body weight supported: animals walk faster

The ability of insects to show walking movements when placed above a slippery surface has been demonstrated in a number of studies (Epstein and Graham 1983; Graham and Cruse 1981; Tang and MacMillan 1986; Tryba and Ritzmann 2000). Graham and colleagues first examined preparations in stick insects in which the weight of the animal was supported (similar to the method used in the present experiments) and friction with the substrate was minimized (Epstein and Graham 1983; Graham and Cruse 1981). They noted that prolonged bouts of walking could be elicited, as in the present experiments. Epstein and Graham (1983) also reported that the animals made no apparent attempts to compensate for leg slipping (such as repositioning or repeatedly flexing the legs within single steps) but instead merely showed a general increase in the rate of walking. We also found that cockroaches with their body weight supported walked at somewhat higher rates (2-4 Hz) than freely moving animals (1-3Hz), although these rates overlapped in range. We should note that these speeds are considerably slower than those seen in running, which in cockroaches can reach 20-25 steps per second (Delcomyn 1985; Full and Tu 1991).

Furthermore, the ratio of stance duration vs. rate of stepping in glass plate walking could be fitted to the curve of freely moving animals. Thus, the apparent effect of reducing weight in these preparations is to merely produce faster walking. It is important to note that the duration of stance largely determines the rate of walking in insects and other animals (Delcomyn 1985; Grillner 1975). If a leg slipped and the duration of stance decreased (Hanson et al. 1999), it would appear the animal was walking faster, provided that the duration of swing remained relatively constant. In the present study we did not systematically examine the exact ranges of leg angles or foot positions and so could not determine if the stride length was shortened during walking on the glass plate. Further studies are planned on the kinematics of joint movements in these preparations to better determine the effects of reduced load upon leg movements.

In addition, animals walking on the vertical oiled surface did not show repeated attempts to grasp the substrate (Larsen et al. 1997) or searching movements (Tryba and Ritzmann 2000a, b). Instead the animals behaved largely as if the substrate were horizontal. The reduction of load borne by the legs and the decreased resistance to leg movement by the slippery substrate may contribute to this finding. Wendler (1966), in early studies of stick insects, found that orientation to the gravitational field in climbing was absent when the body weight was supported. Most insects lack specialized systems for gravity orientation, such as a vestibular (Macpherson and Inglis 1993) or statocyst apparatus, and are thought to detect the effects of body load via the forces that act upon the legs, potentially aided by afferents from the antennae. Thus, animals may utilize the same pattern in walking on horizontal and vertical glass because proprioceptive inputs from the legs no longer signal the gravitational vector or inertial load when body weight is supported.

Motor activities in walking on the glass plate with weight supported

Although motor activities were not extensively analyzed in the present studies, we found the basic patterns of activation of the trochanteral and tibial extensor muscles were similar both in free walking animals and in reduced weight preparations. The trochanteral extensor motoneuron (Ds) typically became active during the late part of the swing phase (Watson and Ritzmann 1998a, b; Tryba and Ritzmann 2000a, b). The tibial extensor (SETi) often fired at a very low rate immediately following leg contact, but showed an acceleration in firing with a peak toward the end of stance (Krauthamer and Fourtner 1978; Tryba and Ritzmann 2000a, b). This acceleration has been associated with a decrease in the mechanical advantage of the femoro-tibial joint as it extends (Larsen et al. 1997) but has also been shown to be phase linked to the decrease of the slow depressor motor neuron (Ds) firing (Tryba and Ritzmann 2000a, b). While no considerable decreases in motor activities were found to occur as a consequence of reducing the weight supported by the legs, this finding could have been mitigated by the increased resistance provided by the support bar (see below). One difference noted in the present experiments was the apparent enhancement of activities of flexor muscles, that occurred as cross talk when walking vertically on glass. Increases in muscle activities in the swing phase have been demonstrated in climbing of locusts (Duch and Pflüger 1995) and may be associated with the force required to lift the leg in the vertical plane.

Forces producing discharges of proximal sensilla: muscles act as levers at the start of stance

The proximal bursts were often of higher maximum frequency during the first one third of the stance phase in walking on the glass plate. An increase in afferent firing could readily result from contractions of leg muscles that would normally support and raise the body being opposed by the harness. The insect body undergoes a repeated raising and lowering from the substrate during walking (Full and Tu 1991; Cruse et al. 1993). Lifting of the body occurs during the time following leg placement upon the substrate and the exertion of vertical forces. While the harness did not impede leg movements and joint excursions, the resistance provided by the support bar above the animal could lead to an augmentation of the strains that occur in the leg from forces exerted in the vertical plane, which would normally be limited only by the animal's weight. These additional forces could occur even though the levels of activity in leg muscles were not increased. Further experiments are planned using a force plate to measure the vertical force component to test this hypothesis.

While the discharges of the sensilla can apparently result from forces generated by the animal, the specific muscles that produce afferent firing may be diverse. We have shown that the trochanteral extensor motoneuron (Ds) is active prior to the onset of stance (Delcomyn 1973; Watson and Ritzmann 1998a) in walking upon the oiled glass, consistent with its contribution to the early discharge. However, other muscles such as those of the body-coxa joints or the tibial flexor may also contribute to the development of strains that excite the proximal sensilla (Alsop 1978). A number of body muscles have been shown to depress the leg during walking in other insects (Duch and Pflüger 1995) although these have not been characterized in cockroaches.

Sensory discharges are reduced in the middle one third of stance

The firing of receptors during the middle one third of stance was significantly decreased in reduced weight vs. free walking preparations. There are a number of factors that could contribute to this finding. First, our data on the patterns of leg coordination in freely moving animals showed that the adjacent mesothoracic and contralateral metathoracic legs are lifted in swing during this period. The increase in force that would occur during this time is absent in animals walking with weight supported. Second, the leg is sliding on the surface so that the resistance to retraction and forces that would be needed to propel the animal's body forward are absent. The contribution of propulsive, horizontal forces to sensory discharge is supported by incidental observations we have made on these preparations. Stepping occasionally produced longer bursts of proximal sensilla toward the end of a sequence or after repeated walks of the animal on the same surface (data not shown). We attribute this effect to drying out and increased viscosity (Epstein and Graham 1983) or rubbing off of the oil. The presence of these bursts supports the idea that muscle activities can generate the afferent discharge. Lastly, however, the increase in rate of walking could contribute to reduced firing at this time as the durations of proximal bursts decrease in freely moving preparations as they walk more rapidly (Zill and Moran 1981b). This effect could be due to an increase in the forces exerted by the legs earlier in stance and to the advance in phase of extensor activity that occurs at more rapid rates of stepping (Delcomyn 1973). However, this decrease in relative duration is only marked at rates greater than 6 steps/second (see Zill and Moran 1981b, Fig. 8). Further experiments, potentially using oils of different viscosities are needed to distinguish the relative contributions of these diverse effects to the reduced duration of bursts seen in preparations walking on oiled glass.

Information provided by force receptors in cockroach walking

Although few studies have examined activities of limb proprioceptive sense organs during walking, the findings of the present investigations are consistent with recent observations on force detection in vertebrates and invertebrates. Experiments that have monitored ground reaction forces during walking show a gradual increase in the forces exerted by a single leg that precede the lifting of other legs in swing. Antigravity muscles are activated prior to the onset of foot placement in a number of animals (Grillner 1975). The extensor muscles fire in cats and cockroaches to break forward motion of the limb in swing but these contractions can also act to press the leg against the substrate after contact (Pearson 1972; Gorassini et al. 1994). This factor could contribute to the finding of the present study that activities of the leg extensor muscles did not show a decrease in mean firing frequency in walking with the body weight supported. Other studies have demonstrated that extensor activities can change according to the load applied to the leg, as when animals drag a weight (Pearson 1972) or when walking while holding a light Styrofoam ball (Delcomyn 1973). The sustained levels of motoneuron firing in the present study could readily be due to the fact that the extensor muscle contractions act to use the leg as a lever to lift the weight of the body. The rigidity of the support bar would oppose these contractions and effectively mimic a resisting load.

Furthermore, in cockroaches, there has been no demonstration of significant increases in the frequency of extensor motoneuron firing at the onset of stance in slow walking (although fast motoneurons can be recruited in running; Watson and Ritzmann 1998a, b). These increases might be expected to occur if loading were suddenly applied after leg contact. In a recent study of walking in the cockroach Blaberus, Tryba and Ritzmann (2000a) found that the extensor motoneuron (Ds) had a ‘characteristic decline’ in firing rate after leg contact, relative to its activity at the end of the swing phase. This finding implies that this extensor muscle does not show changes that would reflect activation by force-receptor-mediated reflexes.

These patterns of sensory and motor activities are consistent with the idea that the firing of load receptors could sustain the discharges of the extensor muscles rather than being responsible for their initiation by reflexes upon foot contact (Prochazka 1996). This conclusion was also drawn in the ‘foot-in-the-hole’ experiments, in which cats stepped upon a trap door that was suddenly released (Gorassini et al. 1994). The extensor myograms showed a peak prior to foot placement and the difference upon dropping into the hole was that the subsequent components of muscle activities were greatly reduced or absent.

Overall these findings imply that the signals provided by force receptors can reflect the forces generated by muscles in support and propulsion. We should note that the discharges of the receptors could still tune muscle activities to leg loading later in stance. Finally, we suggest that generating forces in advance of loading may be equally advantageous in the control of walking machines that are modeled upon insect mechanisms in producing the smooth and coordinated transfer of loads among legs that are seen in cockroaches.

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CHAPTER 3

WALKING ON A ‘PEG LEG’: EXTENSOR MUSCLE ACTIVITIES AND SENSORY FEEDBACK AFTER DISTAL LEG DENERVATION IN COCKROACHES

SUMMARY

Previous studies in insects demonstrated that leg coordination changes following complete ablation of distal limb segments. However, normal coordination was restored when small ‘peg leg’ prostheses were attached to leg stumps to permit substrate contact. We have adapted this paradigm to preserve appropriate leg mass and inertia, by severing all nerves and muscle tendons in the femur of the cockroach hind leg and converting the animal’s own limb into a peg leg. Recordings of muscle activities and leg movements before and after the denervation showed that: 1) the ‘peg leg’ is actively used in walking and regular bursts occur in motoneurons to leg extensor muscles; 2) driving of motoneuron activity is sufficient to produce ‘fictive’ bursting in a muscle whose tendon (apodeme) is cut in the ablation; 3) similar motoneuron activities are found in walking on an oiled glass surface, when the effects of body weight and mechanical coupling are minimized. When distal segments were completely severed in these preparations, leg use and muscle bursting were disrupted but could be restored if the stumps were pressed against the substrate. These results support the hypothesis that feedback from receptors in proximal leg segments indicating forces allows for active leg use in walking.

INTRODUCTION

Walking in both vertebrate and invertebrate animals is thought to be produced by a pattern generator in the central nervous system that is strongly dependent upon sensory feedback (Duysens et al. 2000). Signals from sense organs provide the system with information about leg movements and forces that is essential in maintaining adequate support and generating propulsion (Prochazka 1996). Limiting afferent feedback by limb ablation or denervation can disrupt leg use (Wetzel et al. 1976), while reinforcing sensory signals can aid in restoring walking following central nervous system lesions (Harkema et al. 1997). Many of the same sensory inputs that affect the timing and magnitude of muscle activation within a leg may also provide cues necessary for leg coordination in locomotion (Ting et al. 1998).

In insects, some of the earliest studies on walking tested the effects of sensory feedback upon coordination of leg movements by partial limb ablations and the addition of prostheses (‘peg legs’) (von Buddenbrock 1921; Wendler 1964, 1966; review: Delcomyn 1985). We have performed studies to further examine the contribution of local and intersegmental effects of leg sense organs in ‘peg leg’ animals. In von Buddenbrock’s studies (1921), both middle legs of stick insects were partially amputated (in the femur). The remaining limb stumps had intact muscles and sense organs, and were capable of movement, but they were too short to contact the substrate in posture or walking. These ablations produced immediate changes in the pattern of coordination of the remaining intact legs (as judged by visual observation). Prior to the ablation, animals walked using a tripod gait in which 3 legs are lifted from the substrate simultaneously, leaving a base of support provided by the front and hind legs of one side and a middle leg of the opposite side. After middle leg ablation, coordination changed to a ‘diagonal’ gait, in which the front and hind legs of one side alternated. These changes were thought to be due to lack of sensory inputs from the amputated legs. Subsequent work by Wendler confirmed those findings and found that after attachment of small wooden pegs to the ends of the stumps, stick insects walked with the same gaits as intact preparations (Wendler 1966). These results further suggested that receptors in the ablated leg were responsible for determining the changes in gait. However, removal of one type of receptor that monitors joint angles in the legs, the hair plates, did not cause the change in coordination.

Experiments in cockroaches, utilizing restraint of legs and recordings from leg flexor muscles, suggested that these effects were most likely due to receptors that detected forces in the limbs (Pearson and Iles 1973). If proximal leg segments were restrained so that the foot (tarsus) could not touch the substrate, and the distal joints were free to move, animals showed patterns of coordination similar to preparations in which the leg was ablated in the femur. Furthermore, during walking, the restrained leg could show multiple flexions within single cycles of movement of the unrestrained legs. Recordings of activities of the trochanteral flexor muscle, in preparations in which the femur was severed close to the trochanter, also exhibited multiple bursts within single cycles of movement of other legs (Steel 1979). Pearson and Iles briefly noted that if the stumps were left long enough to make contact with the substrate, the normal pattern of flexor bursting was obtained (Pearson and Iles 1973). The afferent signals in the leg stump that produced these effects were considered to most likely arise from receptors that monitored forces in the leg.

Motor activities in extensor muscles of leg stumps have also been extensively studied (Delcomyn 1988, 1990a,b). Recordings from the trochanteral extensor muscle showed that after ablation of the leg in the mid femur, multiple bursts (double and triple bursts) occurred in the ablated leg within single cycles of movement of intact legs. Multiple extensor bursts were absent during rapid running. However, the activities of leg extensor muscles have not been systematically recorded in ‘peg leg’ animals to test whether bursting occurred after limb contact that could generate support and propulsion.

Thus, data from previous studies suggest the following hypotheses on the original observations of von Buddenbrock and Wendler: after leg ablation, the addition of the peg leg allows for sensory feedback to occur that permits sustained forces to be developed by leg muscles in the stump. This could allow the peg leg to be used in support and propulsion and subsequently permit the resumption of coordination in patterns similar to intact animals. The present study was therefore undertaken to record activities of leg extensors in a peg leg preparation. We utilized a new methodology in which leg nerves and muscles are severed in the femur, rather than ablating the leg and attaching a prosthesis. This lesion eliminates distal sensory and motor innervation and utilizes the animal’s own leg as the peg leg. This method, therefore, avoids potential increases in weight and inertia that could be produced by a prosthesis. Furthermore, we also tested the effects of denervation in animals that walked upon an oiled glass surface with body weight supported (Noah et al. 2001). These preparations walk readily but mechanical coupling of legs and the effects of body weight are minimized. However, animals that walk with weight supported can still show bursts of activities from receptors that monitor leg forces due to the resistance provided by the support bar.

The present studies therefore address the questions 1) are peg legs being actively used in walking?; 2) do muscle activities depend upon coupling through the substrate and body weight upon the leg? Our results support the idea that the prosthesis used in the peg leg experiments allowed muscles in the limb stump to generate forces after limb contact sufficient to provide support and propulsion, resulting in normal leg coordination.

METHODS

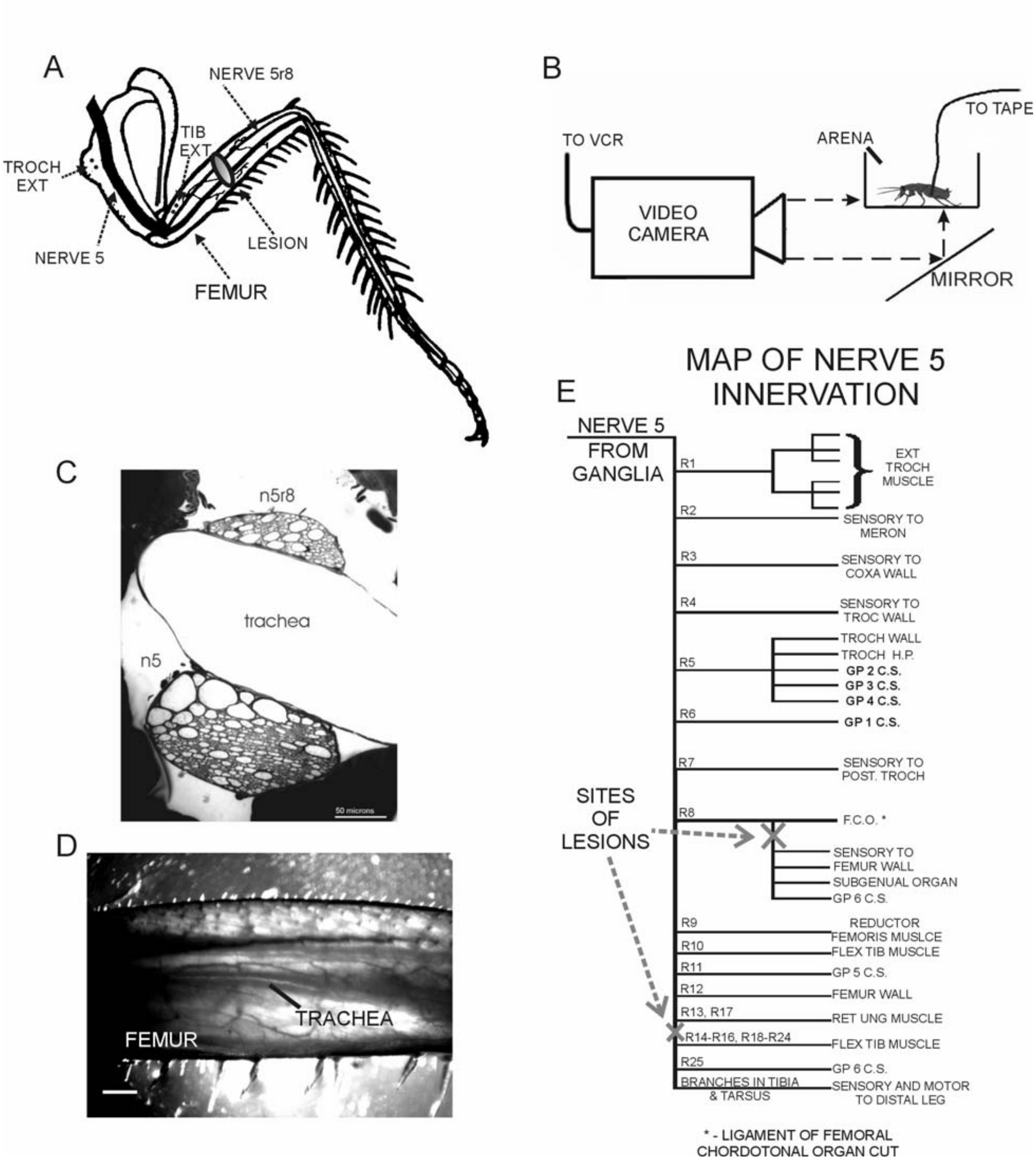
Free-walking preparations

Animals were first restrained on a resin-coated platform and pairs of fine wire electrodes were inserted into the left hind leg to record discharges from either the trochanteral extensor or the tibial extensor motor nerves (Fig. 14A). All recording wires were tied to the leg and secured with cyanoacrylate adhesive. Small dots were painted in pairs on the leg segments of the coxa and femur with white fingernail polish (Wet ‘N Wild) to allow for subsequent digitization of the coxo-trochanteral joint angles in video images. In some studies, dots were also placed on middle legs to monitor protraction/retraction movements.

Preparations were then released into an arena that had clear plexiglass walls and a nylon mesh floor (Fig. 14B). Cockroaches often walked spontaneously but slow stepping rates could be elicited by gently stroking the animals with a fine paintbrush. Walking movements were videotaped at either 125 or 250 frames per second using a Redlake high-speed camera. Video sequences were stored on SVHS video tape for subsequent analysis. A mirror set at 45 degrees below the arena allowed for lateral and inferior views of the animal within a single image (Noah et al. 2001). Leg lifting and placement was accurately observed in the side view, while the dots on the legs could be readily seen in the view in the mirror. Because animals walked closer to the substrate after leg denervation, we were rarely able to obtain kinematic data in three dimensions

Figure 14. Experimental Setup and Site of Denervation. A. Activities of leg muscles were recorded in freely moving animals before and after lesion of all nerves and muscle tendons (apodemes) in the femur. Fine wire electrodes were inserted into the leg to record the activities of the trochanteral extensor (TROCH EXT) or tibial extensor (TIB EXT) muscles. The course and branching pattern of the main leg nerve (NERVE 5) are shown within the leg. The trunk of nerve 5, branch 5r8 (NERVE5r8) and all other soft tissues were cut through a small flap in the middle of the femur (indicated by the shaded oval) . B. Experimental setup for data acquisition in freely moving animals. Animals were videotaped as they walked freely in an arena. Video images contained views from the side and from below through a mirror. Motor activities were recorded onto digital audio tape (TO TAPE) and video images were stored on SVHS tape (TO VCR). C. Histological section of femur (transverse to its long axis) showing location of nerve 5 (n5) and ramus 8 of nerve 5 (n5r8). Both nerves course adjacent to the central leg trachea. D. The central trachea (TRACHEA) was visible through the cuticle by trans-illuminating the leg with a fiber optic light source and was used as a landmark for making the lesions. E. Map of branches of Nerve 5 in the cockroach leg (redrawn after Nijenhuis and Dresden 1958). The X's on the diagram indicate the points at which Nerve 5 and 5r8 were lesioned. All axons were severed from sense organs distal to these points in the femur, tibia and tarsus. Lesion of nerve 5 also denervated the tarsal muscles and distal part of the tibial flexor muscle. The lesion was extended to cut the apodemes of the tibial extensor and flexor muscles, the retractor unguis and the ligament of the femoral chordotonal organ. As indicated by the map, this lesion left the innervation of trochanteral sense organs intact , including groups 1-5 campaniform

sensilla (C.S.), as well as the trochanteral hairplate (H.P.). The viability of the sense organs following this lesion has been confirmed by previous physiological studies.


A
NERVE 5r8
TIB EXT
TROCH EXT
LESION
NERVE 5
FEMUR
B
TO VCR
ARENA
TO TAPE
VIDEO CAMERA
MIRROR
C
n5r8
trachea
n5
50 microns
D
TRACHEA
FEMUR
E
MAP OF NERVE 5 INNERVATION
NERVE 5 FROM GANGLIA
R1
EXT TROCH MUSCLE
R2
SENSORY TO MERON
R3
SENSORY TO COXA WALL
R4
SENSORY TO TROC WALL
R5
TROCH WALL
TROCH H.P.
GP 2 C.S.
GP 3 C.S.
GP 4 C.S.
R6
GP 1 C.S.
R7
SENSORY TO POST. TROCH
SITES OF LESIONS
R8
F.C.O. *
SENSORY TO FEMUR WALL
SUBGENUAL ORGAN
GP 6 C.S.
R9
REDUCTOR FEMORIS MUSLCE
R10
FLEX TIB MUSCLE
R11
GP 5 C.S.
R12
FEMUR WALL
R13, R17
RET UNG MUSCLE
R14-R16, R18-R24
FLEX TIB MUSCLE
R25
GP 6 C.S.
BRANCHES IN TIBIA & TARSUS
SENSORY AND MOTOR TO DISTAL LEG
* - LIGAMENT OF FEMORAL CHORDOTONAL ORGAN CUT

from freely moving preparations after leg ablation. Measurements of the coxo-trochanteral joint angle in three dimensions were obtained from some animals in walking on the oiled glass. Neurographic recordings were stored on digital audio tape (TEAC model RD-130TE).

Synchronization of kinematic and neurophysiological data was based upon an accessory, strobe signal from the camera that produced a pulse at the start of each frame (although continuous illumination was used in these studies). These pulses were recorded on tape and ceased when the camera was turned off at the end of a test. After sequences were digitized in Motus software we aligned the time of the last frame digitized with the last strobe pulse. We were able to confirm the accuracy of this technique by having a parallel method of synchronization. During a test, a hand held switch was used that generated white squares on the video images. These signals were also recorded as a separate channel on tape. We subsequently determined the time of onset of the white squares and found that they aligned with the recorded pulses to a level of accuracy that reflected the frame rate of the camera (ex. 4 msec at 250 frames per second). These methods assured that data on joint angles and leg movements were accurately synchronized with muscle recordings.

Method of denervation and controls for accuracy of ablation

After initial bouts of walking, animals were again restrained and leg nerves were severed to denerverate the distal leg segments. The site chosen for denervation was in the mid femur, based upon the anatomical map of Dresden and Nijenhuis (reproduced in diagram in Fig. 14E) (Dresden and Nijenhuis 1958; Nijenhuis and Dresden 1952, 1956). Cutting nerves 5 and 5r8 at this point eliminates all sensory innervation to the tibia and tarsus, and also denervates the femoro-tibial, tibio-tarsal and distal tarsal joints. We have confirmed elements of this map in anatomical studies (Zill et al. 1993). We have also previously utilized this lesion in physiological studies characterizing activities of the trochanteral campaniform sensilla. We found that all spiking activities from mechanoreceptors of the distal leg were eliminated in recordings of nerve 5, while proximal sense organs remained viable and intact (Zill et al. 1999). To sever the leg nerves, a small incision was made in the middle of the femoral segment, transverse to its long axis (Fig. 14A). In the femur, nerve 5 and branch 5r8 course adjacent to the main trachea of the leg (Fig. 14C). This trachea was used as a landmark to locate the leg nerves, as it was readily visible when the cuticle of the femur was transilluminated with a fiber optic light source (Fig. 14D). Fine tipped iris scissors were inserted into the incision and used to sever the nerves and adjacent trachea. In most experiments we also cut the tendons of the tibial extensor and flexor muscles and extended the ablation sufficient to sever other nerves in the femur (Guthrie 1967). We were able to confirm the accuracy of the lesion at that time by 1) lack of any behavioral response or changes in myographic activities to touching the tibia or tarsus, 2) elevation of the distal tarsus due to cutting the apodeme of the retractor unguis muscle and 3) absence of movements of distal leg segments to mechanical stimulation of the abdomen and cerci. A mixture of paraffin oil and petroleum jelly was placed on the incision to limit bleeding and dehydration of the leg. We were able to bring the margins of the cuticle around the incision into close proximity with a small hook. Animals were then released into the arena and bouts of walking were recorded.

In some experiments, walking movements of preparations that had the leg denervated in the femur were also tested after complete amputation of the distal leg segments. Animals were briefly restrained and the leg was cut in the proximal tibia or in the femur just distal to the incision made for leg denervation. Preparations were then returned to the arena (or oiled glass surface (see below)) and walking episodes were videotaped and recorded.

At the end of an experiment, the left hind leg was removed at the body-coxa joint. The accuracy of nerve lesions was confirmed by dissection of the femur. We were readily able to find the cut ends of both nerves due to their proximity to the main trachea. Data from preparations which had incomplete lesions were discarded. We should note that the cell bodies of one major sense organ that encodes position and movements of the femoro-tibial joint, the femoral chordotonal organ, remained intact following this ablation. Although the ligament of the organ was severed in the femur, effectively eliminating mechanical coupling of the receptors to displacements of the femoro-tibial joint, the sense organs retained the ability to discharge tonically. As those receptors would signal the position indicated by the ligament after it was cut, they could have contributed to some of the variability seen in the activities of the tibial extensor motoneuron (see below).

Walking on oiled glass

To determine if leg coupling through the substrate was essential to walking patterns after nerve lesion, animals were supported above a glass plate coated in microtome oil according to the methods of Noah et al. 2001. A harness that was covered with glue and steel particles was attached to the animal after recording wires were implanted. Preparations were then mounted to a support bar with a magnet on its end that effectively held the harness. The height and orientation of the bar was adjusted until animals displayed spontaneous bouts of locomotor activity. Video images were recorded at 250 or 125 frames per second during walking sequences.

Data analysis

Neurophysiological data were transcribed to digital files with a Cambridge electronics (Spike2) interface system. Walking sequences were stored as digital images from tape using a Matrox RT2000 video capture card. The times of leg lifting and placement were determined from video sequences and stored in a spreadsheet. Data were analyzed using our own Spike2 scripts and Sigmastat software. Histograms were constructed in Sigmaplot.

RESULTS

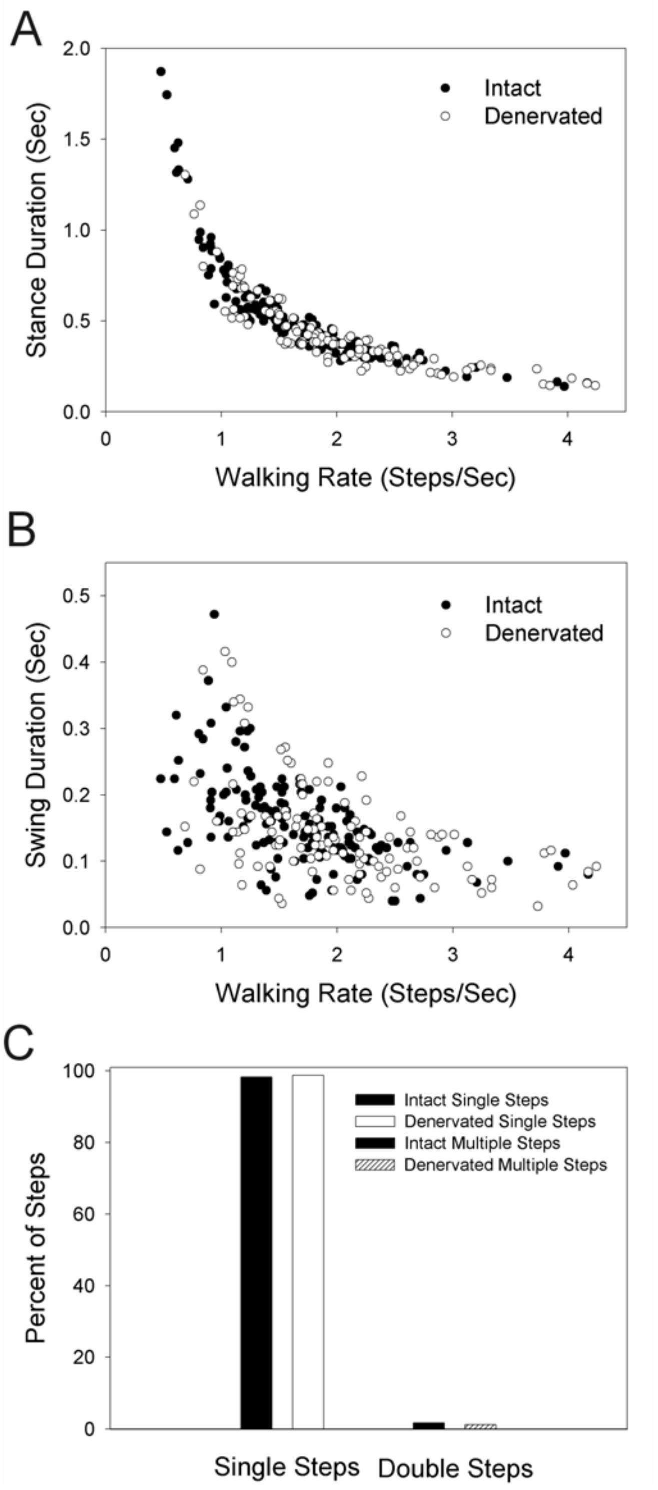
Walking movements after denervation

Animals were able to walk with little difficulty after severing all nerves in the femur of a single hind leg. During the stance phase, most cockroaches leaned toward the side of the ablation after the hind leg was placed upon the substrate. The femoro-tibial joint (a hinge joint) did not collapse under the animal’s weight. The plane of movement of the joint is at an acute angle with the substrate and loads acted nearly in line with the joint condyles (Hughes 1952,1957; Full and Ahn 1995). However, small flexions at the joint may have contributed to the animal’s slight tilting during stance. During swing, the leg was regularly lifted free of the substrate and not dragged along the surface.

We determined the durations of swing and stance from video sequences both before and after denervation (Fig. 15). Figure 15A is a plot of the duration of the stance phase versus the rate of walking before and after denervation (14 animals:151 steps before,117 steps after). The values from both groups overlap and stance phase duration decreases with increasing rates of walking (Delcomyn 1987; Noah et al. 2001). However, we found that the mean values of the duration of stance are significantly different (0.524 +/- 0.28s intact; 0.419 +/- 0.21s denervated, Student's t-test P <0.001). This difference reflects the fact that the animals walked at a somewhat faster rate after denervation (1.72 +/- 0.656 steps/sec in intact and 2.15 +/- 1.061 steps/sec in peg leg animals – significant for P <0.001). Figure 15B shows the duration of the swing phase at different rates of walking from the same walking sequences in Figure 15A. The mean duration of swing for animals is 0.158 +/- 0.07 s before ablation and 0.142 +/- 0.08 s afterwards. These values are not significantly different. Thus, most of the basic parameters of walking were unchanged by leg denervation.

Previous studies found that after partial leg ablation, multiple steps could occur in the ablated leg within cycles of movement of intact legs (Delcomyn 1990a,b). To determine if animals show multiple stepping after denervation, we measured the time of foot contact and lifting of both hind legs. We then counted the number of onsets of swing in the left (ablated) leg within step cycles of the opposite right (intact) hind leg. Figure 15C shows the percentage of single versus multiple steps seen in 7 animals during walking before (58 steps) and after (83 steps) denervation of the leg. The number of multiple steps was very low and occurred both before and after ablation only on rare occasion.

Figure 15. Walking before and after leg denervation. A. Times of swing and stance were determined from video images of leg movements. Plot of stance phase duration versus rate of walking. The duration of stance decreased as the rate of walking increased both before and after denervation of distal leg segments. The data form a continuum although animals tend to walk somewhat faster after the lesion. B. Plot of duration of swing versus rate of walking. The swing phase duration decreased as the rate of walking increased up to 2Hz but remained relatively constant at higher rates of stepping. Similar values were obtained before and after denervation. C. Incidence of multiple stepping. Multiple stepping was determined by counting the number of leg lifts (initiations of the swing phase) of the denervated leg during the single step cycles of the opposite hind leg. If legs were lifted more than once, it was considered a multiple step. Multiple stepping was rare prior to and after denervation.


A
2.0
1.5
1.0
0.5
0.0
Stance Duration (Sec)
0
1
2
3
4
Walking Rate (Steps/Sec)
● Intact
○ Denervated
B
0.5
0.4
0.3
0.2
0.1
0.0
Swing Duration (Sec)
0
1
2
3
4
Walking Rate (Steps/Sec)
● Intact
○ Denervated
C
100
80
60
40
20
0
Percent of Steps
Intact Single Steps
Denervated Single Steps
Intact Multiple Steps
Denervated Multiple Steps
Single Steps
Double Steps

Patterns of trochanteral extensor activity in walking

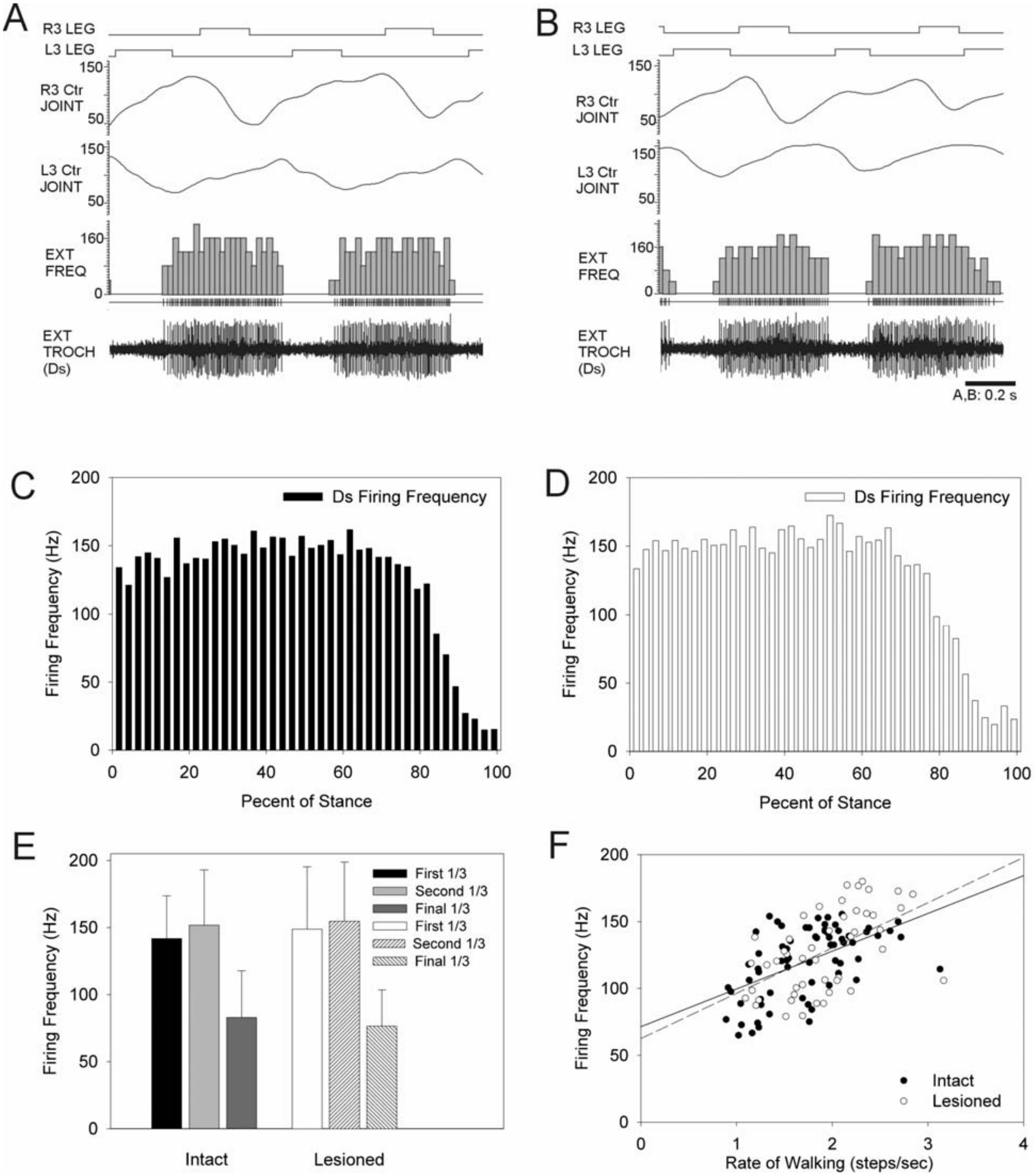
Figure 16 shows activities of the trochanteral extensor motoneuron (Ds) during walking before and after denervation of the distal leg segments. This figure also contains the times of leg lifting and placement, as well as the coxo-trochanteral joint angles for the two hind legs (two-dimensional angles as determined from video images taken from below the animal). In intact animals (Fig. 16A), the trochanteral extensor is active prior to foot placement (Watson and Ritzmann 1998). The extensor continues to fire at a relatively uniform rate through most of stance but its frequency declines in the last one third of the stance phase. The patterns of leg lifting and placement show a strict alternation of swing movements in the two hind legs. This is also reflected in the plots of the coxo-trochanteral joint angles that extend during stance and decrease during swing in antiphase in the two hind legs. The same characteristics of motoneuron activity and alternation of leg movements were seen after denervation (Fig. 16B). However, in this sequence the coxo-trochanteral joint in the ablated leg was used in a more extended range after the denervation and was not flexed below 100 degrees. These changes in the range of movement of the coxo-trochanteral joint did not occur in all preparations but may be attributable to mechanical factors associated with the ablation. After severing the tendons of the tibial extensor and flexor muscles, the femoro-tibial joint angle could change passively and be forced into extension when the leg was moved by the action of the proximal leg muscles. As a consequence, the inertia of the leg increased and it was not moved through its normal range at the more proximal joint. These changes in ranges of movement did not produce systematic changes in motoneuron activities or coordination with the opposite hind leg after denervation.

Normalized histograms of motoneuron firing frequencies in the stance phase were constructed to quantitatively compare motor activities before and after denervation. Figure 16C is a histogram showing mean Ds activities during the stance phase (divided into 40 bins) in 68 steps of 7 animals when walking at a mean rate of 1.73 +/- 0.49 Hz. Figure 16D is a comparable histogram of the same animals walking at approximately the same rate after nerve ablation (1.90 +/- 0.46 Hz, total = 47 steps). Ds firing occurred at similar mean rates both before and after the denervation. Other studies have shown Ds firing frequency can be modulated at different points in the step cycle (Cruse et al. 1993; Watson et al. 2002). We, therefore, divided the stance phase into thirds and calculated the mean Ds firing frequency during each period of stance (Fig. 16E). There were no significant differences found in Ds firing frequency during any individual third of stance in animals before and after ablation. Figure 16F shows a plot of the mean Ds firing frequency versus the rate of walking. The mean Ds firing frequency increases as the rate of walking increases (Watson and Ritzmann 1998) and a similar increment is seen after denervation. Thus, the basic characteristics of bursting of the trochanteral extensor were preserved after the ablation.

Patterns of tibial extensor activity in walking

Bursting activity persisted in the slow tibial extensor motoneuron (SETi) after the distal leg segments were denervated and the tendons of the tibial extensor and flexor muscles were cut. Figure 17 shows recordings and plots of activities of the extensor both before and after denervation of the distal leg. The SETi motoneuron is active in

Figure 16. Activities of the trochanteral extensor motoneuron in freely walking animals. Discharges of the trochanteral extensor were recorded during walking before (A) and after (B) denervation of the distal leg, and synchronized with data digitized and measured from video images. A. Bursts of the extensor [EXT TROCH (Ds)] were initiated before foot placement (L3 LEG). Firing continued at a sustained level in stance and produced extension of the coxo-trochanteral joint (L3 CTr JOINT). Movements of the two hind legs were in antiphase as indicated by the joint angle (R3 CTr JOINT) and foot fall patterns (R3 LEG). B. Similar motoneuron activities and leg movements were seen after denervation, although the CTr joint range was further in extension after the ablation. C,D. Normalized histograms of Ds activity in the stance phase before (C) and after (D) lesion of distal leg. Trochanteral extensor firing frequencies were sampled based upon the times of leg placement and lifting and stance was divided into 40 bins. The firing frequency of the motoneuron was similar both before and after denervation. E. Comparison of Ds firing frequencies at different periods in stance. The stance phase was divided into thirds and the mean discharge rate calculated for each interval. No significant differences were found in Ds firing during any part of the stance phase (Student’s T-test). F. Plot of mean Ds firing frequency in stance vs. rate of walking. The mean firing frequencies showed similar increases at faster rates of walking before and after the lesion.


A
R3 LEG
L3 LEG
R3 Ctr JOINT
150
50
L3 Ctr JOINT
150
50
EXT FREQ
160
0
EXT TROCH (Ds)
B
R3 LEG
L3 LEG
R3 Ctr JOINT
150
50
L3 Ctr JOINT
150
50
EXT FREQ
160
0
EXT TROCH (Ds)
A,B: 0.2 s
C
Ds Firing Frequency
Firing Frequency (Hz)
0
50
100
150
200
Pecent of Stance
0
20
40
60
80
100
D
Ds Firing Frequency
Firing Frequency (Hz)
0
50
100
150
200
Pecent of Stance
0
20
40
60
80
100
E
First 1/3
Second 1/3
Final 1/3
First 1/3
Second 1/3
Final 1/3
Firing Frequency (Hz)
0
50
100
150
200
Intact
Lesioned
F
Firing Frequency (Hz)
0
50
100
150
200
Intact
Lesioned
Rate of Walking (steps/sec)
0
1
2
3
4

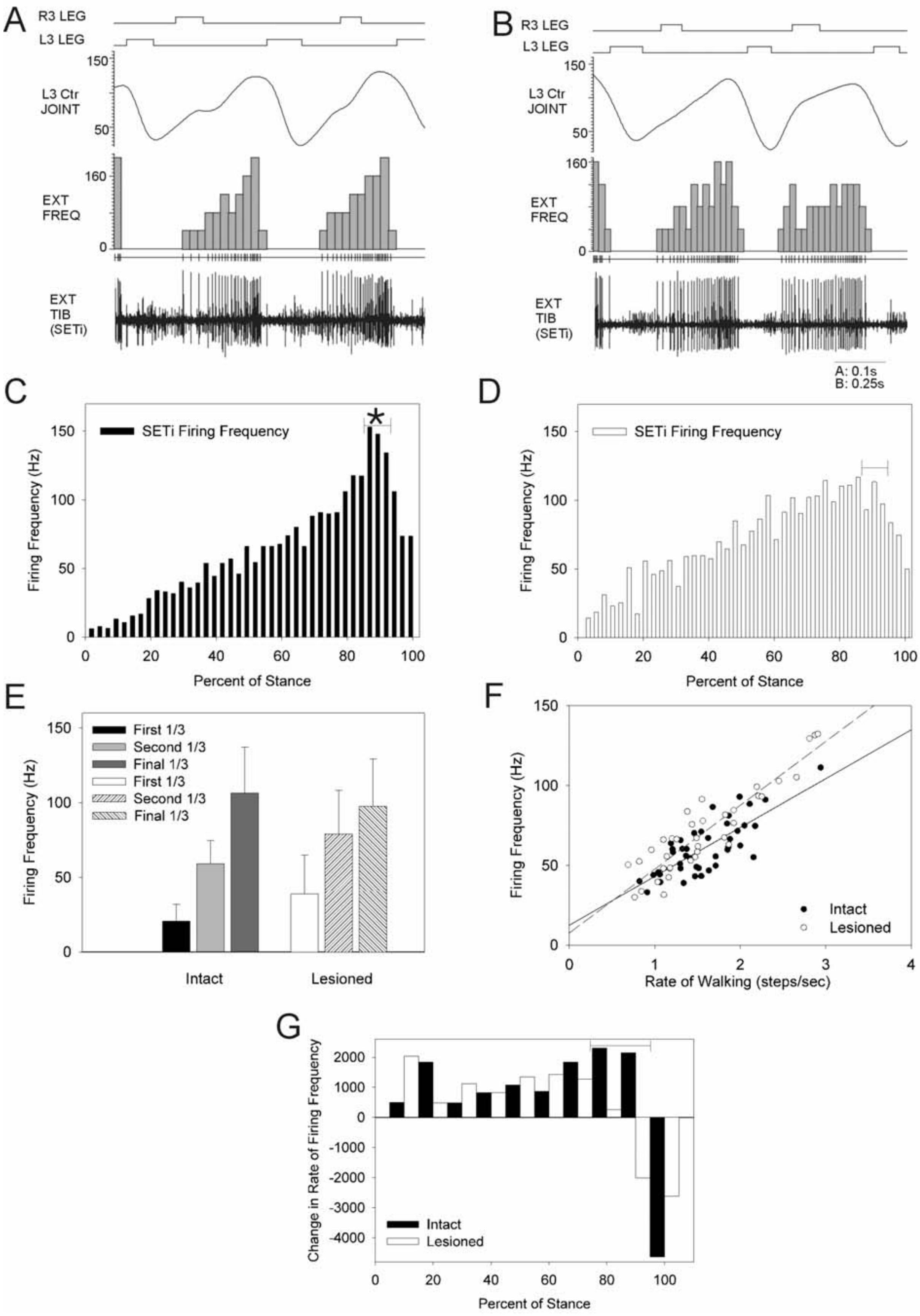
stance in a characteristic pattern: firing is initiated at a very low rate after leg contact and accelerates through the stance phase (Krauthamer and Fourtner 1978). In addition, there is often a discrete higher acceleration present near the end of stance prior to leg lifting (Fig. 17A) (Watson and Ritzmann 1998; Tryba and Ritzmann 2000).

After the ablation, bursting of the slow extensor persisted in all preparations but was more variable in its pattern of discharge. This variability was present both between individuals and in different walking sequences of single animal. On average, some of the characteristics of extensor firing were still present after the ablation, while others were altered. In the recording in Figure 17B, the firing of the SETi motoneuron shows an overall slow acceleration in the burst during the stance phase. An increment in discharge rate during a burst was present during slow walking in all animals tested, although not in every step. However, the firing frequency reaches a higher level earlier in stance. This higher discharge rate was often sustained and the final acceleration near the end of the burst was not apparent.

The consistent differences between extensor discharges before and after the ablation are reflected in the cumulative histograms shown in Figures 17C and 17D. As the firing frequency of the tibial extensor depends upon the rate of walking, (see Figure 17E below) (Watson and Ritzmann 1998, Tryba and Ritzmann 2000) these histograms were constructed from walking sequences taken in the same range of rates (1-3 steps/second, N=3 animals). The gradual increment in firing is present in both histograms but the final acceleration, which forms a prominent peak in Figure 17C (bins 35-37 in the histogram) is absent in Figure 17D. There is a significant difference in the mean firing frequency in these bins after ablation (137.655 +/- 43.651Hz intact; 109.050

Figure 17. Effects of denervation on activities of the slow tibial extensor motoneuron (SETi) in freely walking animals. A. The tibial extensor (EXT TIB SETi) typically became active some time after foot placement on the substrate (L3 LEG) and the firing frequency of the motoneuron increased throughout stance. The extensor also showed a final acceleration of discharge prior to the swing phase. Extensor firing occurred during the time of extension of the coxo-trochanteral joint (L3 CTr JOINT) which moved in antiphase with the opposite hind leg (R3 LEG). B. 'Fictive' burst in the slow extensor after denervation of the distal leg and cutting the tibial extensor and flexor apodemes. After denervation, activity of the extensor motoneuron was variable but showed a similar gradual acceleration of activity in a burst. The firing frequency was higher early in stance and the peak of activity at the end of the burst was less apparent. Bursting still occurred during the period that the coxo-trochanteral joint angle extended and swing movements alternated in the two hind legs (L3 LEG, R3 LEG). C,D. Normalized histograms of SETi activity in the stance phase during walking at 1-3 steps/second before (C) and after (D) denervation of the distal leg. Both histograms show an acceleration of firing during the extensor burst. Firing early in stance was somewhat higher after the ablation. The acceleration prior to the onset of swing (asterisk) is not apparent after the ablation (bins indicate by bar are significantly different). E. Comparison of SETi firing frequency before and after the ablation when data in stance was divided into three periods (as in Fig. 16E). After the ablation, the slow extensor discharged at a higher rate in the first two thirds of stance but the mean firing frequency was not significantly different in the last third, as the extensor fired at a plateau after the ablation. F. SETi firing frequency at different rates of walking. The slow extensor

showed a similar increment in frequency as the rate of walking increased, both before and after lesion of leg nerves. G. Rate of change of extensor firing. The interspike intervals were used to calculate the rate of change of extensor firing within a burst. This histogram displays the change in the rate of SETi firing before and after leg denervation when data in stance were divided into 10 bins. The rate of change in firing frequency is maximal at the end of stance before the ablation, but decreases during this time after leg denervation.

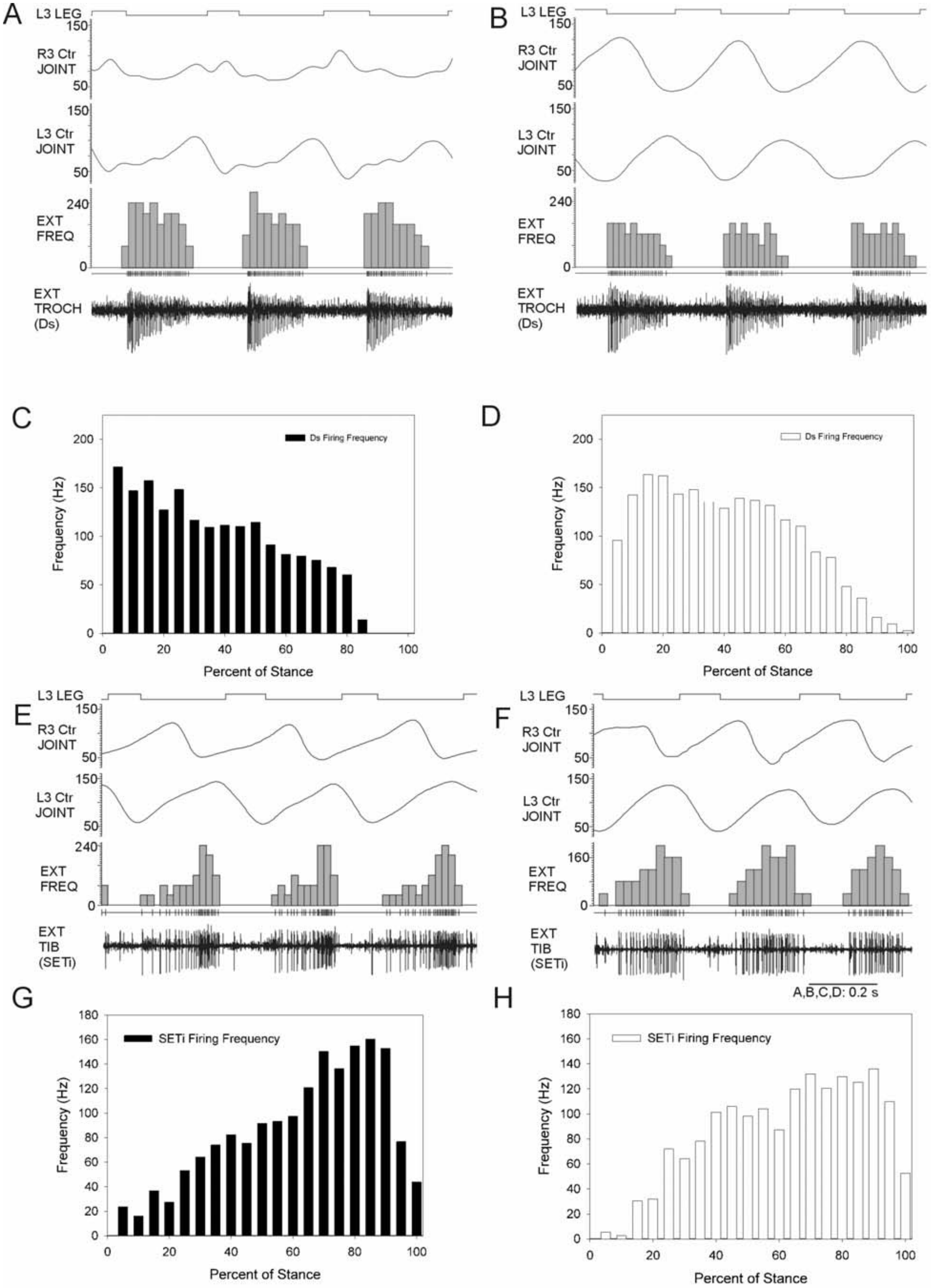

A
R3 LEG
L3 LEG
L3 Ctr JOINT
150
50
EXT FREQ
160
0
EXT TIB (SETi)
B
R3 LEG
L3 LEG
L3 Ctr JOINT
150
50
EXT FREQ
160
0
EXT TIB (SETi)
A: 0.1s
B: 0.25s
C
SETi Firing Frequency
*
Firing Frequency (Hz)
0
50
100
150
Percent of Stance
0
20
40
60
80
100
D
SETi Firing Frequency
Firing Frequency (Hz)
0
50
100
150
Percent of Stance
0
20
40
60
80
100
E
First 1/3
Second 1/3
Final 1/3
First 1/3
Second 1/3
Final 1/3
Firing Frequency (Hz)
0
50
100
150
Intact
Lesioned
F
Firing Frequency (Hz)
0
50
100
150
Rate of Walking (steps/sec)
0
1
2
3
4
Intact
Lesioned
G
Change in Rate of Firing Frequency
2000
1000
0
-1000
-2000
-3000
-4000
Intact
Lesioned
Percent of Stance
0
20
40
60
80
100

+/- 35.716 ablated; Student's t test, P = 0.002). To further characterize this difference in SETi bursting we calculated the rate of change of firing frequency for the slow extensor (Fig. 17G). In intact animals the rate of change of frequency increases as the joint extends, after an initial rise at the onset. After the ablation, the highest rates of change occur earlier in stance and the peak at the end of stance is absent. This is also reflected in Figure 17F, which plots the mean firing frequencies in each period after stance is divided into thirds (Noah et al. 2001). During the first two thirds of stance the SETi firing frequency is significantly higher after the ablation (20.636 +/- 11.296Hz, 59.179 +/- 15.526Hz intact; 40.103 +/- 27.060 Hz, 80.227 +/-29.987 Hz ablated; both significant at P <0.001). During the final third of stance, however, there is no significant difference in the mean discharge rate (106.295 +/- 30.899Hz intact; 99.60 +/- 33.785Hz ablated). Figure 17E is a plot of the overall mean firing frequency of the extensor at different rates of walking. The increment found in the first two thirds of stance produces a slightly higher mean discharge rate, although the dependence of the firing frequency upon the rate of stepping is preserved after the ablation.

Motor activities during walking on an oiled glass plate with body weight supported

We further examined the effects of leg denervation in animals that walked while suspended on an oiled glass surface (Tryba and Ritzmann 2000; Noah et al. 2001). Mechanical coupling between the legs is largely eliminated in these preparations (Epstein and Graham 1983). Our previous studies have shown that the effect of body weight on the legs is substantially reduced when animals are mounted in the harness (estimate 80% reduced, Noah et al. 2001). Figure 18 shows recordings of activities of the trochanteral extensor motoneuron in walking on oiled glass before (Fig. 18A) and

Figure 18. Motoneuron activities while supported above a slippery surface. A,B. Recordings of the trochanteral extensor motoneuron during walking while supported over an oiled glass surface. The trochanteral extensor fired bursts during the stance phase before (A) and after (B) denervation of the distal leg. In both recordings, firing is initiated before foot placement (L3 LEG) and there is a strict alternation of movements of the two hind legs (L3 CTr Joint, R3 CTr Joint). The decline in amplitude of the motoneuron was associated with slight movements of the muscle relative to the recording electrodes. C,D. Normalized histograms of the mean Ds firing frequency in stance during the sequences shown in A and B (stance divided into 20 bins). The discharge was higher at stance onset and a decline in Ds frequency occurs later in stance in comparison with activity in freely moving animals. However, similar results are obtained before (C) and after (D) denervation (C: 15 steps at walking rate 1.97 +/- 0.18Hz, mean firing frequency = 107.02 +/- 8.76 Hz; D: 16 steps at 2.27 +/- 0.28 Hz mean firing frequency = 118.96 +/- 13.6 Hz). E,F. The tibial extensor also fired regularly in stance in walking on oiled glass and showed an overall acceleration of firing both before (E) and after (F) distal leg denervation. The SETi bursts occur during the period when the CTr joint extends (L3 CTr JOINT) and alternates with movements of the opposite hind leg (R3 CTr JOINT). G,H. Normalized histograms of SETi activity constructed from the sequences show in E and F (20 bins). The gradual acceleration is present before and after the denervation of the leg, but firing is increased early in the burst following denervation (G: rate 2.20 +/- 0.21 steps/sec, mean frequency = 88.65 +/- 9.60 Hz; H: 12 steps at 2.49 +/- 0.30 steps/sec, mean frequency = 102.51 +/- 7.88 Hz).


A
L3 LEG
150
R3 Ctr JOINT
50
150
L3 Ctr JOINT
50
240
EXT FREQ
0
EXT TROCH (Ds)
B
L3 LEG
150
R3 Ctr JOINT
50
150
L3 Ctr JOINT
50
240
EXT FREQ
0
EXT TROCH (Ds)
C
Ds Firing Frequency
Frequency (Hz)
200
150
100
50
0
0
20
40
60
80
100
Percent of Stance
D
Ds Firing Frequency
Frequency (Hz)
200
150
100
50
0
0
20
40
60
80
100
Percent of Stance
L3 LEG
E
150
R3 Ctr JOINT
50
150
L3 Ctr JOINT
50
240
EXT FREQ
0
EXT TIB (SETi)
L3 LEG
F
150
R3 Ctr JOINT
50
150
L3 Ctr JOINT
50
160
EXT FREQ
0
EXT TIB (SETi)
A,B,C,D: 0.2 s
G
SETi Firing Frequency
Frequency (Hz)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Percent of Stance
H
SETi Firing Frequency
Frequency (Hz)
180
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Percent of Stance

after (Fig. 18B) the ablation. Figures 18C and D are cumulative histograms of activities in stance from these sequences that are constructed by the same methods as Figures 16A and 16B. The Ds motoneuron shows regular bursting in the stance phase at similar frequencies both before and after the denervation. Double bursting of the trochanteral extensor, as is found in movements of stumps of severed limbs, did not occur nor was multiple leg lifting observed during stepping of the ablated leg. The histograms show that, in this sequence, the mean firing frequency was higher at the start of the burst and declined earlier than in freely walking animals. These differences occurred both before and after leg denervation and may be attributed to the specific body height at which walking was elicited (Cruse et al. 1993) and the fact that the effects of body weight are reduced (Noah et al. 2001).

The changes in firing of the tibial extensor motoneuron that occurred in free walking preparations were also apparent in tests of stepping on oiled glass. Figures 18E and 18F are recordings of the extensor motoneuron from an animal walking supported over the slippery surface before and after ablation. Figures 18G and 18H are cumulative histograms of firing during the stance phase from these sequences. Prior to the denervation, the tibial extensor motoneuron showed both the gradual increase in firing frequency during a burst, as well as a final acceleration of activity prior to leg lifting. After denervation, the firing frequency of the extensor is increased early in the burst, but apparently lacks the acceleration in discharge rate at the end of the stance phase. The increase in activity was reflected in an increment in the mean firing rate of the extensor during stance (88.65 +/- 9.60 Hz before denervation and 102.51 +/- 7.88 Hz after ablation). Furthermore, the ablated leg moved in a pattern of strict alternation with the opposite hind leg, as indicated both by the patterns of leg lifting and placement and by the changes in the coxo-trochanteral joint angles.

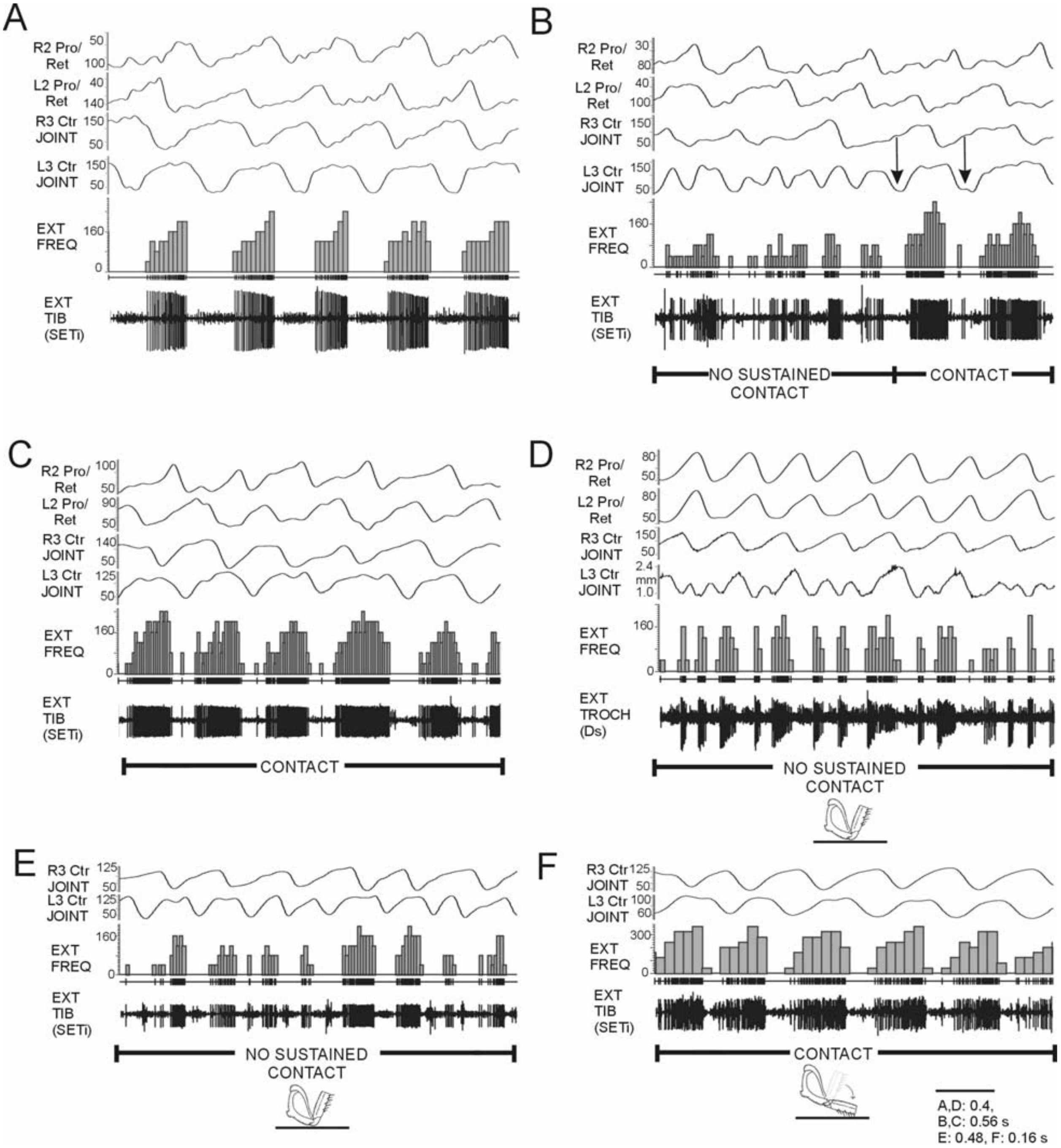
Thus, the preservation of bursting following denervation of the distal leg is not apparently dependent upon mechanical coupling of the legs through the substrate. Similar characteristics of bursting also occur even though the effect of body weight upon the legs has been reduced.

Effects of amputating the denervated leg in the mid femur

We tested whether the regular bursting of extensor motoneurons recorded in denervated legs was dependent upon substrate contact by restraining animals with nerve lesions and completely amputating the distal leg segments in the mid femur. Figures 19A-C show activities of the tibial extensor motoneuron during sequences of walking that were recorded after the initial denervation and following the subsequent removal of the distal leg. The coxo-trochanteral joint angles of the hind legs and the positions of the right and left middle legs have been synchronized with the recording. The sequence in Figure 19A shows the firing of the slow tibial extensor after denervation in a freely walking animal (similar to Figure 17). The mean rate of firing is apparently higher early in stance and an acceleration in firing frequency occurs in each burst. The coxo-trochanteral joint angles show that swing movements in the two hind legs alternate and these changes are in antiphase with the protraction/retraction movements of the middle legs. The pattern of muscle activities and leg movements changed dramatically after severing the leg in the proximal tibia (Fig. 19B). This animal, at first, did not bring the ablated leg into contact with the substrate. However, toward the end of the sequence, the leg was extended sufficiently to press against the floor of

Figure 19. Effects of distal leg amputation and substrate contact. A,B,C. Recordings of slow tibial extensor in a preparation that was first denervated (A) in the mid femur and then distal leg segments were amputated in the proximal tibia (B,C). The traces above show the coxo-trochanteral joint angles of both hind legs (L3 denervated, R3 intact), as well as protraction and retraction movements of the two middle legs (L2, R2). The SETi fired bursts in A that are similar to those seen in Figure 17. The joint angles and leg positions indicate that the L3 and R2 legs moved in phase and alternated with the R3 and L2 legs. After severing the leg, the stump was at first unable to make contact with the substrate (B, left part) and multiple bursts and flexion/extension movements occurred in the ablate stump. When the stump was extended sufficient to make contact (arrows in B), prolonged extensor bursts occurred and regular coordination was restored. The animal made repeated contact with the stump in a succeeding walking episode (C) and bursting occurred that was similar to that seen following distal leg denervation. D. Activity of the trochanteral extensor from the preparation shown in Figure 18A,B in walking on oiled glass after ablating the leg in the distal femur. The animal was unable to make contact with the substrate at any point in this sequence. The motor recording indicates irregular, repetitive bursts of activity while the coxo-trochanteral joint angle shows multiple flexion extension movements in single cycles of the opposite hind leg. E. Activity of tibial extensor from the preparation shown in Figure 18E,F after amputation of the distal leg. The ablated leg (L3) often showed multiple stepping during cycling of the opposite hindleg (R3). F. Walking episode from the same animal as E in which the stump makes contact with the substrate during each

step. Motor bursting was similar to that seen after denervation and no multiple steps occurred in this sequence.


A
R2 Pro/ Ret 50 100
L2 Pro/ Ret 40 140
R3 Ctr JOINT 150 50
L3 Ctr JOINT 150 50
EXT FREQ 160 0
EXT TIB (SETi)
B
R2 Pro/ Ret 30 80
L2 Pro/ Ret 40 100
R3 Ctr JOINT 150 50
L3 Ctr JOINT 150 50
EXT FREQ 160 0
EXT TIB (SETi)
NO SUSTAINED CONTACT
CONTACT
C
R2 Pro/ Ret 100 50
L2 Pro/ Ret 90 50
R3 Ctr JOINT 140 50
L3 Ctr JOINT 125 50
EXT FREQ 160 0
EXT TIB (SETi)
CONTACT
D
R2 Pro/ Ret 80 50
L2 Pro/ Ret 80 50
R3 Ctr JOINT 150 50
L3 Ctr JOINT 2.4 mm 1.0
EXT FREQ 160 0
EXT TROCH (Ds)
NO SUSTAINED CONTACT
E
R3 Ctr JOINT 125 50
L3 Ctr JOINT 125 50
EXT FREQ 160 0
EXT TIB (SETi)
NO SUSTAINED CONTACT
F
R3 Ctr JOINT 125 50
L3 Ctr JOINT 100 60
EXT FREQ 300 0
EXT TIB (SETi)
CONTACT
A,D: 0.4,
B,C: 0.56 s
E: 0.48, F: 0.16 s

the arena (indicated by the arrows as noted from video images). Early in the sequence, when the leg does not touch the substrate, there are multiple bursts in the slow extensor and repeated small flexion/extension movements during single steps of the opposite hind leg. In contrast, prolonged bursts occur in the tibial extensor when the leg makes contact with the surface during the last two steps. Figure 19C shows a subsequent episode of walking in which the animal is able to maintain contact with the substrate in each step. During this sequence, there are no multiple leg movements or motor bursts. The motor activity closely resembles that seen before amputation of the distal leg (Fig. 19A).

We also tested the effects of severing the distal leg upon walking when mounted above the oiled glass. Figure 19D shows a recording of the trochanteral extensor (from the same animal as shown in Figure 16) after severing the leg in the femur in walking upon the slippery substrate. The animal was unable to make contact with the walking surface at any point in this sequence. The motor recording shows multiple bursting while the coxo-trochanteral joint angle clearly displays repeated small extensions and flexions within the cycles of movement of the R3 leg. Figures 19E and 19F show recordings of the slow tibial extensor in an animal that walked suspended over a slippery surface after complete amputation of the distal leg (in the femur). Multiple bursts occurred in the extensor when the animal was not able to make contact with the substrate (6E) but strong bursting and 1:1 coordination occurred when the animal extended the stump sufficiently to make sustained contact with the surface in stance (6F). These results strongly support the idea that signals for contact and sustained force development allow the extensor muscles in limb stumps to show relatively normal bursting, despite denervation or loss of the distal leg.

DISCUSSION

We have utilized a new ‘peg leg’ preparation, in which distal segments of the hind leg are denervated but remain attached to the animal, to study the roles of sensory feedback in walking. We have found that motoneurons to the trochanteral and tibial extensor muscles show vigorous bursting during walking, both in freely moving animals and in preparations that walk when supported above a slippery surface. These findings strongly suggest that the ‘peg legs’ can be actively used in walking and that motor outputs do not depend upon mechanical coupling of leg movements through the substrate. We have also found that extensor firing is abbreviated and multiple bursts occur in single step cycles if the denervated segments are completely amputated and the remaining leg stump does not contact the substrate. However, regular bursting, similar to that seen in a denervated leg, is immediately restored if the stumps can be pressed against the walking surface. In the following we first discuss the method of denervation of the distal leg, then review the properties of motoneuron bursts that are preserved or altered after denervation and finally discuss how inputs from sense organs in the ‘peg leg’ can contribute to the patterns of muscle activities and leg coordination seen in walking.

Effectiveness of lesion in denervating the distal leg and effects upon walking movements

We produced a complete denervation of sense organs and muscles of the distal leg by making an incision in the mid femur and cutting the main trunk of nerve 5 and its branches, the tibial extensor and flexor muscles and their apodemes, as well as all surrounding soft tissues. The site of the lesion is similar to the location where the leg was ablated in the original peg leg experiments of Wendler (Wendler 1964). It was also advantageous as the major nerve branches are grouped around the central trachea and could be reliably severed through a small opening (Dresden and Nijenhuis 1958; Nijenhuis and Dresden 1952, 1956; Zill et al. 1993). Furthermore, previous studies have shown that the sense organs of the trochanter remain viable following this lesion (Zill et al. 1999) and could signal both forces applied to the leg and the position of the coxo-trochanteral joint. Preparations also walked readily after the lesion and the femoro-tibial joint did not collapse (completely flex) when supporting the animal's weight, even though it could not be actively moved after the apodemes of the tibial muscles were severed. The plane of movement of the joint is at an acute angle with the substrate to produce forward propulsion by the hind leg extensor muscles (Hughes 1952,1957; Full and Ahn 1995). Consequently, the weight of the animal acts perpendicular to the hinge articulation and thus produces only limited forces in the joint plane. However, the resulting small movement that occurs may have contributed to the animal’s tilting toward the side of the ablation during walking.

Activities of the trochanteral extensor

Recordings of activities of the trochanteral extensor (Ds) during walking of freely moving animals showed similar patterns of activation both before and after severing all nerves and muscle apodemes in the femur, when the distal leg segments remained attached to the limb. Motoneuron firing was initiated before foot placement and was maintained at an equivalent, sustained level during stance, following denervation of the distal leg segments (Watson and Ritzmann 1998). The mean firing frequency also showed a linear relationship with the rate of stepping that was unchanged (Delcomyn 1987). These findings suggest that the afferent inputs that remain intact in the proximal leg segments are sufficient to provide sensory feedback necessary for the generation of activities of the trochanteral extensor in slow walking. The sense organs of the trochanter have been demonstrated to be a primary source for proprioceptive feedback to mediate these effects on the Ds motoneuron (Zill et al. 1999). Activation of the extensor prior to the onset of stance is thought to be mediated by afferents of the trochanteral hairplate that discharge during leg flexion (Wendler 1966; Pearson et al. 1976) and can monosynaptically excite the Ds motoneuron. These effects are thought to function to limit joint flexion in swing and stiffen the joint prior to foot placement and leg loading (Noah et al. 2001). Sustained Ds bursting during the stance phase can be aided by the trochanteral campaniform sensilla (Pearson 1972). These receptors are strongly excited by forces applied to the leg that mimic leg loading and have also been shown to produce short latency excitation of the trochanteral extensor (Zill et al. 1999). Experiments utilizing a small device to deform the trochanter in freely moving stick insects have shown that the leg is held in an extended position during walking when the cuticle is continuously stressed. These results imply that the effects of the trochanteral campaniform sensilla on motor output are present in freely moving animals (Bassler 1977). Thus, the sense organs of the proximal leg provide the system with information both about joint movements and force that can modulate and sustain the trochanteral extensor in walking.

The Ds motoneuron also fired regular bursts in stance after distal leg denervation when animals walked while supported over a slippery surface. This finding implies that mechanical coupling between legs is not necessary for bursting in these preparations. Although extensor activities were more variable in walking on oiled glass, firing at equivalent frequencies occurred both before and after section of peripheral nerves. Variability in Ds firing was also found when stick insects walked while supported over a slippery surface (Epstein and Graham 1983), and may be related to the height of the animal above the substrate (Cruse et al. 1993). In the present experiments, the position of the animal was adjusted until spontaneous bouts of walking were elicited, and the body height at which stepping occurred varied among preparations (as indicated by video images). Furthermore, our earlier experiments using the same apparatus showed that the extent of body weight that is supported by the legs is greatly reduced in these preparations (Noah et al. 2001). This reduction in the effect of body weight could contribute to the variability produced by eliciting locomotion at different body heights. In sum, these results support the idea that the movements of the denervated leg in walking of freely moving animals do not occur passively as a result of the action of other intact legs upon the body, but instead are generated by active contractions of muscles within the 'peg leg'.

'Fictive' bursting of the tibial extensor

Firing of the tibial extensor motoneuron persisted in walking after the denervation of the distal leg, even though the apodemes (tendons) of the tibial extensor and flexor muscles were severed in the mid femur. This activity therefore represents a form of 'fictive' bursting in that it produced no movements of leg segments. The specific pattern of discharge of the slow extensor showed some variability, but similar bursts were also elicited when animals walked on the oiled glass with weight supported. These discharges retained the gradual acceleration of firing within a burst but, on average, firing frequencies were increased early in stance and lacked the final acceleration prior to the onset of swing. This produced a more uniform discharge rate within a burst, similar to that occurring during rapid walking (Krauthamer and Fourtner 1978; Zill and Moran 1981) when the influence of sensory inputs is considered to be reduced (Zill 1985). Firing of the slow extensor at a uniform rate also has been recorded in preparations in which the thoracic connectives are cut (Krauthamer and Fourtner 1978). Bursting in these preparations is thought to be produced by the activities of non-spiking interneurons in the central nervous system that form components of the pattern generator (Pearson and Fourtner 1975). However, 'fictive' bursts after distal leg ablation also retained the gradual acceleration in firing that is present in slow walking, suggesting that some tuning by sensory inputs may also occur. Specific sense organs that could contribute to the increase in firing rate within an extensor burst have not been determined in cockroaches. We did note that the slow acceleration of activity of the tibial extensor can be present after complete amputation of the distal leg, even in the absence of contact with the substrate. One source of patterning of this activity could lie in receptors that encode the angle of the coxo-trochanteral joint, which remain intact in these preparations (Brodfuerhrer and Fourtner 1983; Pearson et al. 1976). Furthermore, the variability that occurred in extensor discharges may also be associated with signals from the afferents of another joint angle receptor, the femoral chordotonal organ. As we have noted, although the ligament of the organ was severed, the chordotonal neurons would still have discharged tonically. This activity may have varied as the position of the ligament was not fixed after the ablation. Sustained changes in the position of the femoral chordotonal organ of stick insects have also been shown to substantially alter levels of activity during motoneuron bursts (Akay 2002). Similar effects of the chordotonal organ could have changed the pattern of extensor discharges seen in the present experiments.

Recent work in stick insects has also shown that a similar acceleration of activity occurs in the tibial flexor muscle, which is active during the stance phase of walking in those animals (Gabriel et al., in press). A group of campaniform sensilla located on the proximal femur has also been shown to have strong excitatory effects upon the tibial flexor in 'active' preparations in stick insects (Akay et al. 2001; Akay 2002). Comparable effects from trochanteral or femoral campaniform sensilla have not been demonstrated in cockroaches. These findings are consistent with the idea that the pattern of tibial extensor firing after denervation may be determined by the activities of central pattern generating elements that are modulated by the remaining, intact leg sense organs.

The discrete, final acceleration of activity at the end of the extensor burst was apparently absent after distal leg denervation. Although it was often difficult to discern in individual steps or walking sequences, a significant difference was found in firing of the extensor in this period in pooled data (Figs. 17C, 17D). The function of this acceleration is unknown, although it has been noted that it occurs at the time of the decrease in firing of the trochanteral extensor (Tryba and Ritzmann 2000). Recordings from the tarsal chordotonal organ in freely moving animals showed that afferent firing of the receptors, which signal lifting of the claws and arolium from the substrate, consistently follows the end of the extensor burst (Larsen et al. 1997). Furthermore, Gorb has recently shown in flies that the detachment of the tarsus prior to swing may involve twisting or pulling of legs away from the substrate (Niederegger and Gorb 2003). These findings suggest that the acceleration of the extensor may aid in release of the tarsus from the substrate in cockroaches. This hypothesis is supported by the finding that the occurrence of a discrete acceleration apparently depends upon afferent inputs from the distal leg segments.

Effects of sensory inputs in ‘peg leg’ walking

The present experiments have demonstrated that regular firing of the trochanteral extensor motoneuron (Ds) occurs during walking after denervation of distal leg segments. These data support the hypothesis on the original observations of von Buddenbrock and Wendler, that animals walking on peg legs can develop sustained forces by contraction of muscles that remain intact in the limb stump. We also found that these bursts persist in walking on oiled glass and can produce vigorous leg movements. Those results demonstrate that muscle activities do not depend upon coupling through the substrate and suggest that they generate sufficient forces to provide (at least in part) support and propulsion in walking. These data also suggest that the movements of the peg leg in animals that are freely walking (and not supported) are active and not merely the result of passive actions produced by movement of the body by other legs. We found that after completely severing the distal limb, multiple bursts could occur in extensor muscles in the ablated leg in single step cycles of other legs, as well as repeated flexion/extension movements, if the stump did not make contact with the substrate. These experiments confirm the findings of a number of earlier studies that have shown that multiple steps occur in leg stumps during slow walking (Steel 1979; Delcomyn 1990a,b). However, more sustained extensor bursting, resembling that seen in denervated legs, occurred if the leg was able to maintain contact with the substrate. These findings also support the basic hypothesis of Wendler (1966) and others, that addition of the peg leg to the leg stump results in the activation of sensory inputs that are absent from the ablated limb without substrate contact.

As we have noted, there are a rich array of sense organs that remain intact after denervation of the leg in the mid femur, including receptors that signal leg position and movement and those that encode leg forces (Zill 1993). It is probable that information from a number of these receptors is utilized in the process of generating prolonged bursts in the stance phase. However, we found that the transition between multiple bursts and prolonged firing could occur in leg stumps within single steps after substrate contact. This finding implies that the cues necessary for the transition could be rapidly incorporated into the motor pattern without more prolonged mechanisms of neuronal or behavioral adaptation. Furthermore, a common factor in these experiments was that sustained bursts were developed when the animal was able to maintain prolonged contact that allowed the muscles to develop forces against the substrate. We consider it likely that receptors that encode forces play an important role in generating these sustained motor activities, as suggested by a number of authors (Pearson and Iles 1973; Delcomyn 1985). Although we have not systematically examined the ranges of movement that occur in walking following denervation, muscle bursting could occur in Ds in some trials in ranges of joint angles that were more extended than those regularly used in walking. This finding implies that similar motor outputs can occur even though kinematic parameters vary. Further studies are planned, using ablations of nerves and sense organs in the peg leg to examine the roles of sense organs of specific modalities in producing forceful extensor muscle contractions.

These experiments support the general idea that ongoing feedback from leg sense organs is an essential component in slow walking. The successful performance of walking movements requires the nervous system to rapidly resolve the problems of maintaining support and balance while generating propulsion. Mechanisms that incorporate information provided by sense organs detecting leg forces and movements may be a common advantage and underlie the remarkable similarities in control of walking in both invertebrates and vertebrates.

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GENERAL SUMMARY AND CONCLUSIONS

The need to support the load of the body is a necessary constraint during both standing and walking. It is thought that interactions of sense organs in the peripheral nervous system with the central nervous system and skeletal muscles are required to generate support in posture and locomotion. This thesis describes the responses of a type of load detecting sense organ, the campaniform sensillum, during changes in body load in the American cockroach (*Periplaneta americana*). Motor responses have also been described which may be influenced by sensory information from groups of campaniform sensilla. The three chapters presented here describe responses of cockroaches to (1) increases in body load during standing, (2) decreases in the effect of body weight on the legs during walking, and (3) ablation of sense organs from the distal limb upon walking. The results of this study indicate there are similarities in the way the detection of load can influence motor activities during walking in both invertebrates and vertebrates. This summary will review the results of the three studies, briefly compare the findings to those of vertebrates and will present ideas for future studies to understand the control of posture and locomotion.

(1) Increasing the effect of body load on the legs results in activities of the proximal group of tibial campaniform sensilla. These sense organs may provide the animal with information regarding the load on the legs. In addition, motor activities in extensor motorneurons can occur in response to changes in loading. The proximal sensilla have known excitatory reflexes to these muscles in restrained animals. If these pathways are also active in standing, it is reasonable to believe that the sensilla could be contributing to motor responses seen during standing. Increasing the loading of legs

through the use of magnets could also be used when coupled with microneurography recording techniques in vertebrates to determine sensory-motor interactions underlying postural control. Recordings of sensory and muscle activities in response to changes in load in vertebrates can be compared to these findings.

(2) Decreasing the effect of body load during walking had the effect of significantly changing the response of the proximal tibial campaniform sensilla in the middle of the stance phase. The responses of the proximal group of tibial campaniform sensilla can indicate the extent of body loading on legs during walking in addition to forces generated by muscle contractions. Activities in the sensilla were significantly different when animals walked freely versus when they walked with the majority of their body weight supported via a harness. The differences seen can not only indicate the animal’s ability to detect the body load, but activities from the tibial receptors can also provide information about muscle force generated at the onset of the stance phase. Swing to stance transitions could be initiated or adjusted in timing by sufficient loading or unloading of a leg signaled by the sensilla. Sufficient loading of a leg can determine the difference between whether a limb is used in support in the stance phase or if it is lifted again if an animal steps in a hole or on an unstable surface. Activities of sense organs that signal limb loading may underlie recent findings in spinal cord patients that show shorter recovery times when subjected to increases in loading during walking (Harkema et al. 1997). Further studies could be designed to look at instantaneous changes in leg loading at specific times during the step cycle to further characterize the effect of activities of sense organs on timing of leg movements during walking.

(3) The third study denervated the distal segments of the leg to determine changes in motor activities seen during walking. Muscle bursting was found to be similar during walking before and after ablation of the distal limb in cockroaches. This denervation had the effect of eliminating sensory activities from the tibial campaniform sensilla, however, proximal sense organs from the trochanter which can also detect forces in the leg remained intact. We amputated the leg in the distal femur so that it was unable to make contact with the walking substrate. An increase in multiple bursting of extensor motoneurons was seen during bouts of walking where the leg did not make sustained contact with the substrate. If the stump of the leg could make prolonged contact with the surface, motor activities were restored to normal. The results support the hypothesis that activities of proximal sense organs are sufficient for producing normal motor activities during slow walking. Further studies should determine the influence of the remaining intact proximal sense organs, including the campaniform sensilla of the trochanter.

The ideas presented in this thesis support previous work, which suggests there are similarities in the control systems for standing and walking in vertebrates and invertebrates (Duysens et al 2000). The studies suggest that force receptors in invertebrates can detect the development of muscle force and the effect of body load on the legs. This is important in understanding how the nervous system decides if a leg will support the load of the body in the stance phase or if the limb will be repositioned in the case of stepping in a hole or on an unstable surface. Future studies could be performed in vertebrates to determine if Golgi tendon organs have similar properties to campaniform sensilla.

ABSTRACT

Sense organs in the legs that detect body weight are an important component in the regulation of posture and locomotion. This thesis seeks to gain an understanding of how body weight is detected by sense organs of the legs, and determine how this information influences muscle activities in standing and walking. The first study tested the ability of tibial campaniform sensilla (receptors that monitor forces in the cockroach leg) to encode variations in body load using magnets attached to the thorax. Recordings of sensory activities in freely standing animals showed that proximal tibial sensilla (oriented perpendicular to the leg long axis) encode the level of body load while distal receptors (oriented parallel) fired to decreasing loads. In some postures, sensillum discharges paralleled changes in activity of the trochanteral extensor muscle consistent with a known interjoint reflex. These findings demonstrate that tibial campaniform sensilla can monitor the effects of body weight upon the legs and may aid in generating support of body load.

In the second study, sensory activities were compared when animals walked freely in an arena, or upon an oiled glass plate with their body weight supported. Sensilla discharges persisted but were abbreviated when body load was reduced. The results suggest that sensory discharges early in stance result from forces generated by contractions of muscles that press the leg against the substrate. Force feedback later in stance may adjust motor output to changes in loading.

In the third study, muscle activities and leg movements were recorded before and after denervation of distal leg segments. Regular bursts occurred in motoneurons to leg extensor muscles following denervation, including ‘fictive’ bursting in a muscle whose

tendon (apodeme) was cut in the ablation. Similar motoneuron activities were found in walking on an oiled glass surface, when effects of body weight and mechanical coupling were minimized. When distal segments were completely severed, leg use and muscle bursting were disrupted but could be restored if the stumps were pressed against the substrate. These results support the hypothesis that feedback from receptors in proximal leg segments, that indicate forces, allow for active leg use in walking.

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