

**RESPONSE PROPERTIES OF TIBIAL CAMPANIFORM SENSILLA
ON THE COCKROACH LEG IN RESTRAINED PREPARATIONS
AND FREELY MOVING ANIMALS**

DISSERTATION

Submitted to the Graduate College

Of

Marshall University

In Partial Fulfillment of the Requirements for

The Degree of Doctor of Philosophy

By

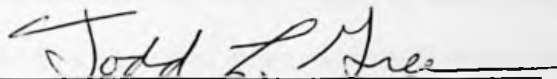
Angela L. Ridgel

Huntington,

West Virginia

2000

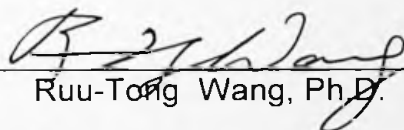
APPROVAL OF EXAMINING COMMITTEE



Todd L. Green, Ph.D.



William B. Rhoten, Ph.D.



Ruu-Tong Wang, Ph.D.



Monica Valentovic, Ph.D.



Sasha N. Zill, Ph.D., Chair

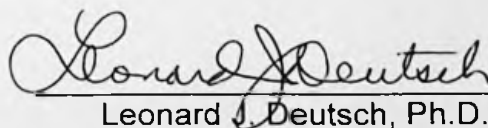
12/4/00

Date

Accepted by Graduate College

12/5/00

Date



Leonard J. Deutsch, Ph.D., Dean

ACKNOWLEDGEMENTS

I would like to express my gratitude to my thesis advisor, Dr. Sasha N. Zill. He has provided unlimited amounts of advice and direction concerning this research and career decisions. I also thank the individuals on my committee: Dr. William Rhoten, Dr. Rudy Wang, Dr. Monica Valentovic and Dr. Todd Green for their support and helpful comments. I am especially thankful to Faith Frazier who has provided instruction, support and friendship throughout my time at Marshall. In addition, I would like to thank David Neff, Laura Quimby and Adam Noah for their advice and help in microscopy, histology and video imaging.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES AND FIGURES.....	vi
INTRODUCTION: FORCE DETECTION IN POSTURE AND LOCOMOTION.....	1
Importance of load detection in posture and locomotion.....	1
Response properties and reflex effects of force receptors	5
Receptors that monitor muscle tension	5
Receptors that encode forces via strains in the exoskeleton.....	8
Summary of responses of force receptors and context of the present investigations.....	13
Problems addressed in the present investigations.....	14
CHAPTER 1	
ACTIVE SIGNALING OF LEG LOADING AND UNLOADING IN THE COCKROACH.....	16
SUMMARY.....	17
INTRODUCTION.....	18
MATERIALS AND METHODS.....	19
RESULTS.....	20
DISCUSSION.....	26
CHAPTER 2	
ENCODING OF FORCES BY COCKROACH TIBIAL CAMPANIFORM SENSILLA: IMPLICATIONS IN DYNAMIC CONTROL OF POSTURE AND LOCOMOTION....	31
SUMMARY.....	32
INTRODUCTION	33
MATERIALS AND METHODS.....	36
RESULTS	41
Identification of sensory units	41

Responses to 'staircase' stimuli	42
Responses to ramp and hold stimuli	46
Effects of pre-loading upon amplitude sensitivity	50
Response of sensilla to changing rates of force application.....	53
Effects of pre-loading upon rate sensitivities	56
DISCUSSION	60
Comparison with previous studies on leg campaniform sensilla	63
Directional sensitivity of the tibial campaniform sensilla	63
Encoding of static load	65
Encoding of rate of applied forces	66
Effects of pre-loading	68
Differences in responses of proximal and distal sensilla	70
Comparison with campaniform sensilla of insect wings	71
Comparison with other force receptors	72
Potential benefits of information about force dynamics in control of posture and locomotion	73

CHAPTER 3

RESPONSE PROPERTIES OF TIBIAL CAMPANIFORM SENSILLA STUDIED BY SUBSTRATE PERTURBATIONS IN THE FREELY MOVING COCKROACH	76
SUMMARY	77
INTRODUCTION	78
MATERIALS AND METHODS	81
Freely moving animals	81
Restrained preparations	90
RESULTS	91
General description of responses to platform perturbations	91
Sensilla responses to platform movement	94
Threshold and response latencies	98
Directional sensitivities of the tibial sensilla	99
Sensilla response to the rate of perturbation	102

Responses to forces 'parallel' to the tibial axis: tests in restrained preparations	109
DISCUSSION	112
Behaviors of animals during substrate displacements	112
Directional sensitivities of proximal and distal subgroups	114
Encoding of forces in lateral and parallel perturbations	115
Variability in sensory discharge frequencies	116
Encoding the rate of platform movement	120
Differences in responses of proximal and distal sensilla	120
Sensory modalities activated during platform perturbations	121
GENERAL SUMMARY	123
ABSTRACT	126
REFERENCES	128

LIST OF TABLES AND FIGURES

Table 1	59
Figure 1	21
Figure 2	24
Figure 3	27
Figure 4	37
Figure 5	43
Figure 6	47
Figure 7	51
Figure 8	54
Figure 9	57
Figure 10	61
Figure 11	82
Figure 12	87
Figure 13	92
Figure 14	96
Figure 15	100
Figure 16	103
Figure 17	107
Figure 18	110
Figure 19	117

INTRODUCTION: FORCE DETECTION IN POSTURE AND LOCOMOTION

This dissertation describes the response properties of force receptors on the cockroach (*Periplaneta americana*) leg in both restrained preparations and freely moving animals. Receptors that measure forces on the legs have been shown to play an important role in the control of posture and walking (review, Duysens et al. 2000). The sense organs that are examined in these studies monitor force via strains in the insect exoskeleton. Strains are small changes in length (formally, the ratio between the change in length and the original length) that result from stresses (force per unit area) in a structure (Alexander 1992; Serway 1996). It is, therefore, possible to monitor the forces acting on a leg in posture and locomotion by measuring the strains that occur in the animal's exoskeleton.

This work is divided into three chapters that present separate studies describing (1) the afferent signals that occur during loading and unloading of a leg, (2) the encoding of force dynamics and implications in the control of posture and locomotion and (3) the responses of force receptors during postural perturbations in freely moving animals. Each chapter includes an introduction that reviews the relevant literature concerning the detection and regulation of forces during postural support and walking. This overall introduction will discuss the nature of forces acting on the legs and body and how they are detected as a background to the specific questions addressed in subsequent chapters.

Importance of load detection in posture and locomotion

A number of studies have shown that the legs of diverse animal species perform similar functions in posture and locomotion (Full et al. 1991; Blickhan and Full 1993). In

order to maintain support while standing, the legs of an animal must generate forces that oppose gravity (Horak and Macpherson 1996). In addition, postural stability requires that the body's center of mass be contained within the base of support provided by the legs (Fung and Macpherson 1999; Horak and Macpherson 1996). Quadrupeds and hexapods are relatively stable when standing because the area supported by the legs is large and the center of mass is near the ground. In contrast, bipeds are fairly unstable because the area of support is small and the center of mass is high (Horak and Macpherson 1996). However, both groups of animals maintain erect postures by contracting appropriate groups of leg muscles (typically extensor muscles) to generate forces to support the body.

Recent experiments have shown that the leg forces are oriented in specific directions and show ongoing variations in magnitude, even when an animal is standing still. For example, the forces that the feet exert upon the ground (known as ground reaction forces) have been measured using force plates in quadrupeds during standing (Macpherson 1994, 1988a,b; Jacobs and Macpherson 1996; Fung and Macpherson 1999). Force plates typically consist of a flat surface that is mounted on supports. Strain gauges are attached to the supports and measure the forces exerted on the plate. To maintain upright posture, cats exert forces with the forelimbs that are directed forward and outward while those of the hindlimb are directed backward and outward (Macpherson 1988a). Furthermore, during standing the body is never completely motionless and forces acting on the legs change as the center of mass is shifted within the base of support (Horak and Macpherson 1996).

Macpherson and colleagues have extensively examined the dynamic regulation of force by perturbing posture in freely standing animals (cats, Macpherson 1988a,b; 1994, Macpherson and Fung 1999; humans, Nashner 1976,1977; Nashner et al. 1979; Dietz et al. 1992; Dunbar et al. 1986; Macpherson et al. 1989). In a paradigm that has been used extensively, cats stood upon a platform that was suddenly and rapidly displaced (100-200 ms duration) (this general method will be utilized in experiments described in the third chapter) (Macpherson 1988a,b). Force plates were used to monitor the forces generated by the legs and the activities of a number of limb muscles were recorded. When the support surface was translated in the horizontal plane, the feet moved with the platform while the trunk remained behind due to inertia (Macpherson 1988a). This created a relative displacement between the center of mass and the legs. To compensate for this discrepancy, the animal contracted leg muscles and developed forces to oppose the displacement. The specific groups of muscles that became active depended upon the direction of the displacement (Macpherson 1988b). The magnitude of the muscle contractions also varied with the rate of perturbation and larger forces were generated when displacements were very rapid. The general conclusion of Macpherson's studies was that compensatory reactions to perturbations involve very rapid responses in muscles that are specifically adjusted to the magnitude and rates of forces that are applied. These findings support the idea that the detection of forces in the legs is important to counter instabilities in standing and perturbations.

During walking, forces on the legs are dynamic and change as the limbs move through the phases of swing and stance (Full and Tu 1990,1991). During swing the leg is in the air and moving forward, while it is on the ground and moving towards the rear in

the stance phase (Grillner 1975). During stance, walking animals must generate vertical forces to resist gravity, horizontal forces to help move the body forward and lateral forces to maintain stability (Alexander 1992, Duysens et al. 2000). A number of studies have measured forces produced by the legs during walking using force plates (Blickhan and Full 1987,1993; Full and Tu 1990,1991; Full et al. 1995). The magnitude of forces produced by the legs has been shown to be directly proportional to the body mass. In addition, forces on individual legs decrease as the number of legs used in locomotion increases. Therefore, six-legged animals are able to distribute forces among the legs more than two or four-legged animals (Blickhan and Full 1993; Full et al. 1995). Forces are minimal during the swing phase and much larger during stance. Loading of a leg begins after foot fall, then increases to a maximum, to provide support and propulsion, and decreases prior to the leg lifting in swing (Full and Tu 1990,1991). Qualitatively similar patterns of changes in forces have been measured in animals as diverse as crabs (Blickhan and Full 1987) and mammals (Cavagna et al. 1977). These findings have illustrated that forces on the legs are changing throughout the step cycle, and that they show both increases and decreases during the stance phase. Other studies have shown that forces decrease very rapidly when a leg slips as a result of loss of friction between the foot and substrate (Hanson et al. 1999; McVay and Redfern 1994). Therefore, an animal's nervous system must be able to detect incremental and decremental changes in forces and generate muscle contractions at the appropriate magnitude and rates to establish and maintain stability as well as produce propulsion.

Response properties and reflex effects of force receptors

Many different types of sensory receptors can contribute to the control of posture and locomotion. The following section will briefly review the response properties and reflex effects of force receptors in a number of different animals. Although their morphology is diverse, their response properties show similarities including: 1) sensitivity to the rate of change of force, 2) adaptation to sustained forces and 3) strong reflex effects on leg muscles. In addition, some of these receptors respond to decreases in force. This commonality of response properties and reflex effects reflects the importance of receptors that detect forces on the legs in posture and locomotion.

Receptors that monitor muscle tension

Golgi tendon organs (GTO) are force receptors in vertebrates that consist of sensory endings near the junction of a skeletal muscle and its tendon (Fukami and Wilkinson 1977; Jami 1992; Davies et al. 1995; Prochazka 1996). Each tendon organ is innervated by branches of a single large diameter (Ib) afferent axon. Forces developed by muscle contraction cause deformation of the sensory terminals and excitation of the receptor. Tendon organs respond to both steady levels of muscle tension and changing rates of tension (Fukami and Wilkinson 1977; Houk and Henneman 1967; Jami et al. 1985; review, Jami 1992). The sensory discharge also shows substantial adaptation during sustained stimuli. For example, when the nerve to the muscle is stimulated, the activity of Golgi tendon organs increases to a maximum and then declines to a static level that is maintained as long as the muscle remains tense (Davies et al. 1995). The amplitude of the peak is linearly related to the rate of change of muscle tension and reflects the dynamic component of the response while the sustained discharge encodes

the level of force (Davies et al. 1995). Given that forces acting on the legs vary during posture and locomotion, it is beneficial for Golgi tendon organs to be sensitive to these changes.

It is difficult to characterize the reflex effects of Golgi tendon organs because very few techniques are available to selectively activate their afferent fibers (review, Duysens et al. 2000). However, excitation of the tendon organ afferents via induced muscle contractions has provided insight into how sensory information is integrated into the motor activity of the leg (review, Jami 1992). These studies have presented evidence that tendon organs provide autogenic inhibition to muscles (Houk and Henneman 1967; Houk 1979; review, Jami 1992). In this reflex, activity in the motoneurons to the muscle is inhibited, via an inhibitory interneuron, when a high level of force is detected by the sense organ. This type of negative feedback can be important in limiting muscle tension to prevent damage (Houk 1979). Although this inhibitory pathway has been demonstrated in restrained and anesthetized animals, it is not clear whether such feedback is functionally important under dynamic circumstances in a behaving animal.

A number of studies have shown that autogenic inhibition may be suppressed in walking animals (humans, Dietz et al. 1992, Stephens and Yang 1996, Dietz 1998, review, Dietz 1992; cats, Conway et al. 1987, Pearson and Collins 1993, Heibert et al. 1996, Heibert and Pearson 1999, review, Pearson et al. 1998). These experiments have focused on the function of feedback from Golgi tendon organs during the stance phase. Just after foot touchdown in stance, the activity of the leg extensor muscles rapidly increases (Sherrington 1910). Studies have shown that tendon organ afferents of extensor muscles can reinforce, rather than inhibit, muscle activity during walking

(Pearson and Collins 1993; Prochazka 1996; McCrea 1998; Pearson et al. 1998; Hiebert and Pearson 1999; review, Duysens et al. 2000). Sensory activity during stance may also prevent the initiation of swing (Duysens and Pearson 1980; Conway et al. 1987; Whelen et al. 1995). For example, electrical stimulation of the tendon organ afferents in ankle extensor muscles prolongs the duration of extensor activity and inhibits the flexor muscle burst in a walking spinal cat (Whelen et al. 1995).

It has been postulated that feedback from these receptors insures that swing will only occur after the leg is unloaded and, therefore, plays an important role in the control of locomotion and the prevention of falling. It is important to note that very few studies have recorded directly from the Golgi tendon afferents in freely moving animals to confirm these proposed functions (Loeb 1981; Appenteng and Prochazka 1984). In walking cats, tendon organ discharges increased during muscle contractions (Appenteng and Prochazka 1984). However, the relationship between muscle force and receptor activity could not be determined because the load on leg muscles was not measured in these experiments. In addition, the potential contributions of tendon organs to signals for leg unloading have not been examined in any vertebrate system.

Muscle spindles are receptors in vertebrates that detect muscle stretch and limb displacement (Prochazka 1996). It has been suggested that muscle spindles could act together with Golgi tendon organs to produce excitatory effects on the extensor muscles (Conway et al. 1987; Hiebert et al. 1995). However, the relative contribution of muscle spindles and Golgi tendon organs in a behaving animal is difficult to assess (Hiebert and Pearson 1999). This is clearly an area for further research.

In crustaceans, receptors that are similar to Golgi tendon organs also encode forces in leg muscles. Apodeme receptors (also called tendon receptors) usually consist of 9-15 bipolar neurons located at the cuticular invagination (apodeme) that forms the muscle attachment to the exoskeleton. These sense organs respond when muscle forces increase but the firing rate of the receptor units declines (adaptation) during sustained contractions (Tryba and Hartman 1997). However, apodeme receptors are very sensitive to the rate of change of force development. Tryba and Hartman (1997) suggest that the dynamic sensitivity of these receptors provides feedback concerning changes in muscle force during locomotion, but this has not been tested directly. Studies in restrained animals have shown that apodeme receptors elicit inhibitory reflexes to extensor muscles (Clarac and Dando 1973). This feedback is believed to be important in resisting load encountered during stance (Duysens et al. 2000).

Sense organs that are similar to the crustacean apodeme receptors have also been identified in insects (muscle receptor organs) and are postulated to function in compensation for leg loading (Matheson and Field 1995, Burrows 1996).

Receptors that encode forces via strains in the exoskeleton

Cuticular stress detectors (CSD) and force-sensitive mechanoreceptors of the dactyl (foot) encode strains in the exoskeleton of crustaceans (Zill et al. 1985; Marchand et al. 1995; Libersat et al. 1987a,b). CSD's consist of 20-60 bipolar neurons that innervate an elastic strand that is attached to an area of soft cuticle in the leg (Marchand et al. 1995). These receptors respond to deformation of the pliable cuticle. Recordings in restrained preparations have shown that some CSD afferents are excited when cuticular forces increase ('on' units), while others respond to force decreases ('off' units). In addition,

some units respond to both increases and decreases in force ('on/off' units). The cuticular stress detectors also have a strong sensitivity to the rate of change of force (Klärner and Barth 1986; Marchand et al. 1995).

The reflex effects of cuticular stress detectors have been studied in restrained preparations and their activities have been recorded in freely moving animals (Klärner and Barnes 1986; Klärner and Barth 1986; Marchand et al. 1995; Leibrock et al. 1996a,b). Two types of CSD's have been described, CSD1 and CSD2, which differ in their reflex effects. In general, activity in CSD1 excites depressor motoneurons of the leg and CSD2 receptors stimulate levator motoneurons (Leibrock et al. 1996a,b). In crustaceans, levator muscles act to lift the leg in swing while depressor muscles support the animal during stance. During walking, CSD1 and CSD2 are both active during the stance phase (Klärner and Barnes 1986; Marchand et al. 1995). These reflexes act to increase the stiffness of the leg by co-activating both the levator and the depressors when the weight of the animal is being supported (Leibrock et al. 1996a,b).

In crustaceans, force-sensitive mechanoreceptors are concentrated on the dactyl (foot) of the leg. Each receptor consists of two sensory dendrites located in a canal within the exoskeleton (Schmidt and Gnatzy 1984). The response properties of these receptors have been extensively studied in restrained and freely moving animals (Zill et al. 1985; Libersat et al. 1987a,b). Two types of force-sensitive sense organs have been described. The receptors located in the proximal region of the dactyl fire an intense discharge at the onset of the stimulus followed by activity at a low level (phasico-tonic units). The initial response of the mechanoreceptors encodes the rate of change of force while the sustained discharge can signal the magnitude of force (Libersat et al.

1987a). These sense organs respond to both force increases and decreases in that they fire to bending in one direction and release of force in the opposite direction. Many units also show adaptation to sustained forces. The mechanoreceptors located on the tip of the dactyl are only activated at the beginning of the stimulus (phasic). However, these units respond at a constant firing frequency to all rates of force application and do not encode the rate of change of force (Libersat et al. 1987a).

The reflex effects of force-sensitive mechanoreceptors on the dactyl have been tested in restrained preparations and their activity has been recorded in freely moving animals. Mechanical or electrical excitation of these receptors produces activity in the levator muscles of the same leg that was sustained for the duration of the stimulus (Libersat et al. 1987a). In addition, stimulation of the force-sensitive mechanoreceptors elicits excitatory discharges in the depressor muscles of adjacent ipsilateral legs. During walking, the receptors were excited during the stance phase and silent during swing (Libersat et al. 1987b). These reflexes create a feedback system that could act to limit the forces on the legs during stance and may be important in the coordination of leg movements (Libersat et al. 1987a,b).

Other invertebrates also have force receptors that measure strains in the exoskeleton. In arachnids, slit sensilla are present on the leg either singly or in groups of up to 29 sensilla (lyriform organs) (Barth and Bohnenberger 1978; Bohnenberger 1981). Each slit has a bipolar neuron that inserts onto a thin membrane on the surface of the exoskeleton (Barth 1981). These receptors respond to minute cuticle deformations perpendicular to the long axis of the slit (Barth 1981). Slit sense organs encode a wide frequency range of stimuli (0.1 Hz- 1.0 kHz) and show sensitivity to the

rate of change of force in the exoskeleton (Barth and Bohnenberger 1978; Barth 1981; Bohnenberger 1981; Blickhan and Barth 1985; Seyfarth and French 1994).

The lyriform organs can be excited by manual deflection of a leg joint. Activity of the receptors stimulates muscles that augment leg movement and acts to relieve the strain on the cuticle (synergic reflex) (Seyfarth 1978a,b). These reflexes are believed to be important in reducing forces on the legs which could cause damage or disarticulation of the leg joint during turning or jumping (Seyfarth 1978a). The response properties of slit sensilla have not been directly tested in freely moving animals but strains in the exoskeleton have been shown to increase during the stance phase in walking spiders (Barth 1985).

Campaniform sensilla are force receptors on the legs of insects that respond to strains in the cuticle which result from muscle contraction and external load (Zill and Moran 1981a,b; Zill et al. 1981). There are 11 groups of campaniform sensilla on the cockroach leg including four groups on the trochanter, one on the femur, one on the tibia and a single receptor on each of five tarsal segments (Pringle 1938b). The sensilla are located near leg joints or in areas of muscle attachment where cuticular stresses would be maximal. Each sensillum consists of a bipolar neuron whose dendrite inserts into an ovoid cuticular cap on the surface of the exoskeleton (Moran et al. 1971). The cuticular cap is believed to be the site of stimulus reception (Chapman et al. 1973, Moran and Rowley 1975). The axon of the receptor projects to motoneuron cell bodies in the thoracic ganglia (Zill et al. 1981).

Early work on cockroach campaniform sensilla has shown that the receptors are directionally sensitive and that they respond to compressions perpendicular to the long

axis of the cuticular cap (Pringle 1938b, Spinola and Chapman 1975). For example, the group on the tibia (Group 6 of Pringle 1938b) is unique in that it consists of two subgroups of receptors (proximal and distal) with mutually perpendicular cuticular caps (see Fig. 1). In restrained preparations, the tibial sensilla are directionally sensitive in that proximal sensilla respond to dorsal bending in the plane of joint movement while distal sensilla are excited upon ventral bending (Zill and Moran 1981a,b). The receptors are also weakly, but directionally, sensitive to axial forces and to imposed torques (Zill and Moran 1981a,b; Cocatre-Zilgien and Delcomyn 1999). However, these studies did not directly measure the magnitude of forces applied to the leg or characterize the responses of the receptors to rates of change of force.

Reflex effects of the tibial campaniform sensilla have been previously examined in restrained preparations and the patterns of afferent activities have been recorded in freely moving animals. In restrained animals, the reflex effects depend on the orientation of the cuticular cap of the receptor (Zill and Moran 1981a; Zill et al. 1981). Mechanical stimulation of the proximal sensilla excites the slow motoneurons to the extensor muscles of the tibia and the trochanter and inhibits the slow motoneurons to the flexor muscles (Zill et al. 1981). Stimulation of distal sensilla has the opposite effect of exciting the flexors and inhibiting the extensors. These reflexes provide a negative feedback system, which is believed to be important in the detection of leg loading during posture and locomotion (Zill et al. 1981). Techniques for recording from the tibial receptors in walking animals were designed by Zill and Moran (1981b). These studies showed that the activity patterns of the sensilla and the leg muscles are consistent with the reflex patterns described in restrained animals. In addition, they demonstrated that

the proximal sensilla respond to initial loading of the leg at the beginning of the stance phase, while the distal receptors are activated at the end of the stance. This pattern of activity suggests that the tibial campaniform sensilla function in load compensation and limitation of muscle tensions in behaving animals.

Summary of Responses of Force Receptors and Context of Present Investigations

Receptors that monitor forces are located at muscle tendons (vertebrate Golgi tendon organs, invertebrate muscle receptors) or in the exoskeleton (cuticular stress detectors, lyriform organs, campaniform sensilla). These sense organs have a number of common properties in their responses to force that are of particular relevance to the studies described in this dissertation. For example, many of these receptors encode the level of force but their activity shows substantial adaptation to sustained stimuli (Fukami and Wilkinson 1977; Zill et al. 1985; Libersat et al. 1987a,b). In addition, particular groups of sense organs (cuticular stress detectors, force-sensitive mechanoreceptors) are activated when forces on the legs decrease (Libersat et al. 1987a,b; Marchand et al. 1995) although these types of responses have been less well studied.

A number of investigations have also shown that most force receptors are sensitive to the rate of applied forces (Barth and Bohnenberger 1978; Bohnenberger 1981; Zill et al. 1985; Klärner and Barth 1986; Libersat et al. 1987a,b; Davies et al. 1995; Marchand et al. 1995; Tryba and Hartman 1997). However, very few experiments have examined how adaptation to sustained loads effects the rate sensitivity of force receptors (Chapman et al. 1979; Juusola and French 1995). Increased tonic loading of a leg could readily occur if an animal stood upon a sloped surface or carried a load. Under these circumstances, sensitivities to changing loads would be particularly important to prevent falling. In

addition, most tests of the response properties of force receptors have been applied in restrained preparations and very few studies have been able to confirm that similar responses occur in freely standing or moving animals (Zill et al. 1985; Libersat et al. 1987b; Marchand et al. 1995). In those circumstances, the forces acting on the legs are more complex because the animal is supporting the body weight.

Problems addressed in the present investigations

This dissertation extensively characterizes the response properties of the tibial campaniform sensilla to changes in force in both restrained and unrestrained cockroaches and addresses three major questions: 1) What are the characteristics of receptor responses when forces on the legs are decreasing and how could they be important in the adaptation of posture and locomotion? 2) What are the dynamic properties of responses to increasing forces and how do receptor sensitivities vary after adaptation to static loads? and 3) Are the tibial sensilla activated during perturbations of spontaneously assumed postures in freely standing animals and how does receptor activity vary with the magnitude and rate of platform movement?

The first chapter examines the dynamic responses of individual sensilla, which are excited when forces in the legs decrease. As has been reviewed above, a number of load receptors have been shown to encode increases in force, while few have been shown to signal force decrements. Rapid detection mechanisms of decreases in load could allow an animal to monitor force changes that regularly occur during walking or those that could be indicative of leg slipping and postural perturbations. In order to adapt walking patterns to the environment, the nervous system must be able to monitor the levels and rates of changes of forces under a wide range of loading conditions.

Therefore, the second chapter systematically examines receptor responses to the rate of change of increases in force and cases in which static load levels are changing. Although these studies use restrained preparations, they tested the responses of the receptors under conditions that are similar to those an animal would encounter during locomotion. The third chapter, however, investigates the response properties of the tibial campaniform sensilla in freely standing animals during rapid platform perturbations. Perturbations of the surface can be used to alter forces on the legs in a controlled and rapid manner. These data suggest that campaniform sensilla provide precise and dynamic information about forces that could be utilized to adapt posture and locomotion to varying terrains and unexpected perturbations.

CHAPTER 1

ACTIVE SIGNALING OF LEG LOADING AND UNLOADING IN THE COCKROACH

SUMMARY

The ability to detect changes in load is important for effective use of a leg in posture and locomotion. While a number of limb receptors have been shown to encode increases in load, few afferents have been demonstrated to signal leg unloading, which occurs cyclically during walking and is indicative of slipping or perturbations. Mechanical forces were applied to the cockroach leg at controlled rates and were recorded activities of the tibial group of campaniform sensilla, mechanoreceptors that encode forces through the strains they produce in the exoskeleton. Discrete responses were elicited from the group to decreasing, as well as increasing, levels of leg loading. Discharges of individual afferents depended upon the direction of force application and unit responses were correlated morphologically with the orientation of the receptor's cuticular cap. No units responded bi-directionally. Although discharges to decreasing levels of load were phasic, these bursts could effectively encode the rate of force decreases. These discharges may be important in indicating leg unloading in the step cycle during walking and could rapidly signal force decreases during perturbations or loss of ground support.

INTRODUCTION

The detection and regulation of forces acting upon the body and legs are now considered integral components in the control of posture and locomotion in many animals (Prochazka 1996). In walking, for example, increase in loading after leg contact is signaled by receptors that reflexively adjust the activities of muscles which generate support and propulsion (Pearson and Collins 1993; Bässler et al. 1991). The subsequent decrease in loading at the end of a step is necessary for the initiation of leg lifting in swing (Whelan et al. 1995) and for normal coordination of leg movements (Bässler 1987). The activities of some groups of limb muscles are also strongly correlated with leg unloading during postural perturbations, and decrements in load may be important factors in determining responses to leg slipping (Jacobs and Macpherson 1996; Mcilroy and Maki 1994). However, the specific mechanisms or receptors detecting unloading of a leg have not been identified in many systems (Zill 1993).

We have studied the responses of the tibial campaniform sensilla of the cockroach, *Periplaneta americana*, which detect forces acting on the legs through strains in the exoskeleton (Schnorbus 1971), to determine the specific parameters that the receptors can encode during walking. The responses and locomotor functions of these sense organs have also been incorporated into models and control systems based upon insect walking (Schmitz et al. 1995). Campaniform sensilla of insect legs are known to respond directionally to forces that bend the exoskeleton (Hofmann and Bässler 1986; Delcomyn 1991), such as occur during leg loading. The directional sensitivity of an individual receptor is correlated with the orientation of its ovoid cuticular cap (Spinola and Chapman 1975). The cap, which is embedded in the exoskeleton, is the site of termination of the

sensory dendrite and is thought to be the locus of mechanoelectric transduction (French 1992). The tibial campaniform sensilla are unique in that they form two subgroups (proximal and distal sensilla) with mutually perpendicular cap orientations (Fig. 1B). Each subgroup exhibits discrete responses when forces are applied to the distal tibia with joint movement resisted (Schnorbus 1971). For example, the proximal sensilla respond to bending in the direction of joint extension while the distal sensilla discharge to forced flexions (Zill and Moran 1981). In the present study, we have applied controlled rates and levels of bending to the leg and have found that the tibial campaniform sensilla, as a whole, not only respond to increasing levels of force but also exhibit discrete responses to decrements of force. These responses are consistent with the demonstrated directional sensitivities of the receptors and do not represent 'on-off' or bi-directional responses (Dickinson 1992). Instead, these new results suggest that, for a given direction of force application, different receptors signal loading or unloading according to the orientation of the cuticular cap.

MATERIALS AND METHODS

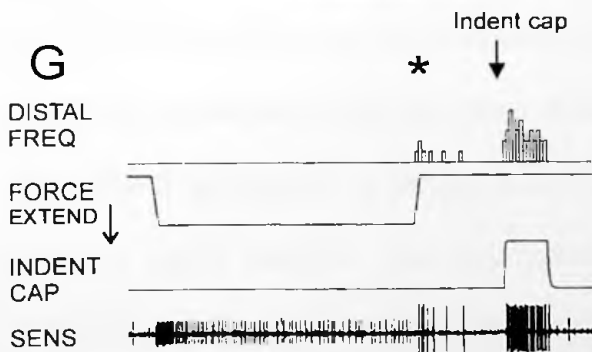
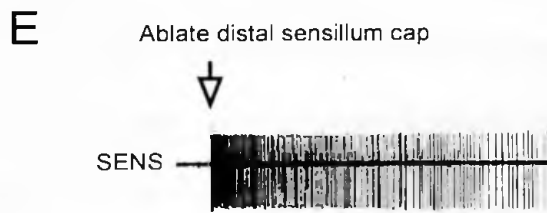
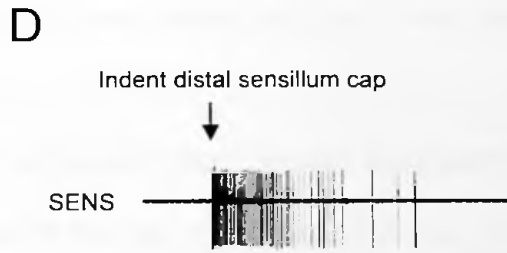
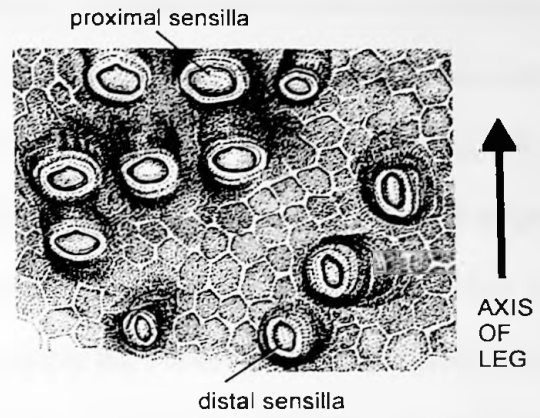
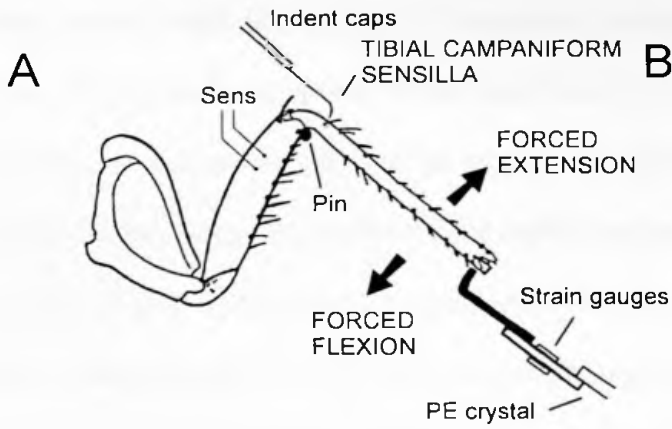
Adult cockroaches, *Periplaneta americana*, (N=21) were anaesthetized with carbon dioxide and the nerves innervating the left metathoracic leg were cut in the thorax. Animals were then restrained with small staples on a resin coated platform. Pairs of fine (50 micron) wires were implanted in the femoral segment of the denervated leg adjacent to the main leg nerve (nerve 5) or its major branch (nerve 5r8) (Nijenhuis and Dresden 1956). These electrodes recorded the activities of both proximal and distal sensilla, as the axons of receptors of both subgroups travel in the same nerve branches (Schnorbus 1971). Typically, the action potentials of one distal and one or two proximal sensilla were evident

in extracellular recordings (N = 17 of 21 experiments) (Spinola and Chapman 1975). The tarsus (foot) and tibial sensory spines were then severed. The femoro-tibial joint was immobilized by gluing a pin adjacent to the proximal end of the tibia with the joint at an angle of 90 degrees or in a position of full extension. Forces were applied to the distal tibia as ramp and hold stimuli via a probe that was driven by a piezoelectric crystal (a complete description of the experimental setup and recording apparatus is found on p. 41). The levels and rates of applied force were monitored through a pair of strain gauges attached to the probe (Fig. 1A). In a typical sequence, we applied 16 different rates, each repeated twice, within a single series and up to three series were repeated in each experiment. In addition, individual campaniform sensilla were identified by indenting their cuticular caps with a fine etched tungsten wire attached to another piezoelectric crystal (Figs. 1A, D) (Spinola and Chapman 1975; Dickinson 1992). All signals were stored on tape for subsequent transcription and data analysis.

RESULTS

Forces applied to the tibia as ramp and hold stimuli elicited discharges both during the rising and the falling ramp phases (N = 16 of 17 experiments in which responses of both proximal and distal sensilla were recorded) (Figs. 1, 2, and 3). In all recordings, the amplitudes of the discharges during the two phases were sufficiently distinct to clearly indicate that different units were active during increasing vs. decreasing levels of load. We performed a number of controls to confirm that these responses originated from the tibial campaniform sensilla and to identify the individual receptors from which the discharges were derived. After testing responses to bending (Fig. 1C), we mechanically stimulated

Figure 1: Preparation and identification of unit discharges to applied forces. **A** Forces were applied to the tibia with joint movement resisted (Pin) via a probe driven by a piezoelectric (PE) crystal. The applied forces (measured in millinewtons, mN; 1mN = 102 mg) were monitored with strain gauges attached to the probe and the activities of the campaniform sensilla were recorded extracellularly (Sens). **B** Drawing of cuticular caps of tibial campaniform sensilla (after Schnorbus 1971). The receptors are located in two subgroups (proximal and distal sensilla) which differ in their directional sensitivity according to their cap orientation. **C** Bending to forced extension (down on the FORCE trace) elicited discharges to different units during force increases and decreases (asterisk). **D** Individual receptors were identified by indenting their cuticular caps (Indent Caps in 1A) with another probe (arrow) which produced a discharge of equivalent amplitude. **E-F** Ablating individual receptors (hollow arrow in E) could selectively eliminate recorded responses to force decreases (hollow asterisk in F). **G** Bending to forced extension (FORCE) was followed rapidly by indentation of the cuticular cap (arrow) of a distal sensillum. **H** Coincident cap indentation (arrow) and decline of force application (asterisk) produced a summation in the discharge of the distal sensillum.

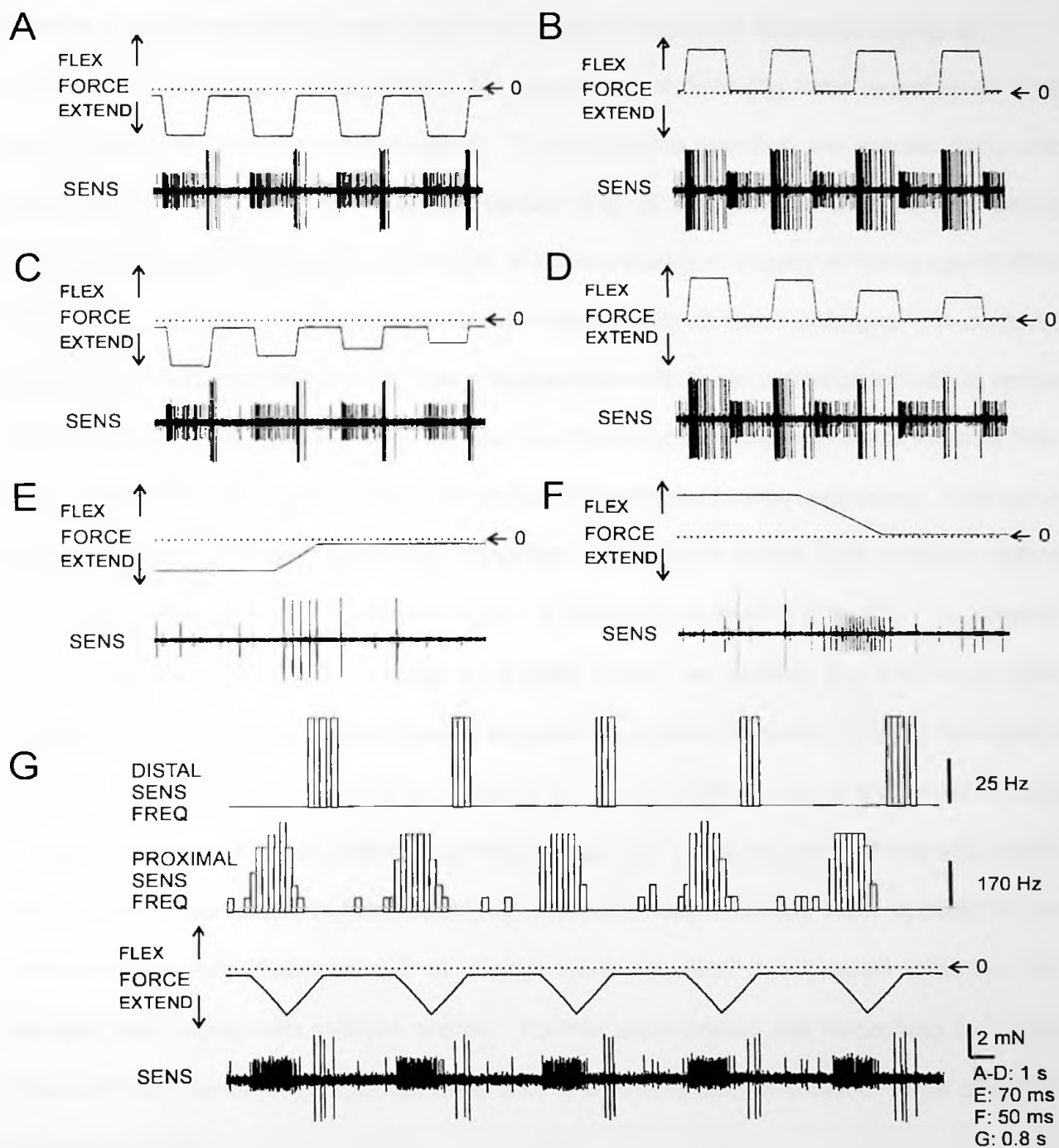


C: 1 mN
F: 0.65 mN
C,F: 0.2 s
D,E: 0.23 s
G,H: 0.1 s

individual receptors by indenting their cuticular caps with a separate probe (Fig. 1D) and were able to elicit discharges of equivalent amplitude in extracellular recordings to those seen during declining levels of bending force. In most experiments the sensillum was then ablated by increasing the level of indentation until the probe penetrated the cap (Fig. 1E). These ablations could eliminate the entire response to decreasing force levels in a single direction while leaving the discharges to force increases intact (Fig. 1F). Furthermore, in three preparations, we were able to simultaneously apply leg bending and cap indentation, which could show a summation during the responses to declining forces (Figs. 1G, H). Thus, the discharges we recorded to bending were clearly derived from the tibial campaniform sensilla.

The responses of individual afferents to decreasing force levels depended on the direction of force application and the orientation of the receptor's cuticular cap. The distal sensilla fired to decrements in the level of forced extension (Figs. 2A, C, E, and G) while the proximal sensilla were active during decreases in forced flexion (Figs. 2B, D, and F). This general pattern occurred stably over time (Figs. 2A, B) and at a variety of levels of displacement (Figs. 2C, D) in repetitive bending tests. Discharges to force decrements were initiated during, and not following, the declining phase of the ramp stimulus (Figs. 2E, F) and were, therefore, not the result of resonance in our apparatus or the consequence of inadvertent application of forces in the opposite direction. We also tested responses to bending using patterns that approximated the durations and magnitudes of ground reaction forces that have been measured during cockroach walking (Full and Tu 1991). Forces applied in the direction of extension as simple, rapid triangle functions, without a

Figure 2: Responses to ramp stimuli applied in different directions. **A** and **C** Bending forces applied in the direction of joint extension (down on the FORCE trace) elicited discharges of proximal sensilla to force increases and distal sensilla to force decreases. **B** and **D** Bending the tibia in the opposite direction (forced flexion, up on the force trace) caused excitation of distal sensilla during force application and proximal sensilla during force declines. **E** and **F** Discharges to decreasing forces occurred during the declining ramp and were not due to rebounds in the opposite direction of bending (zero level = no bending force applied) or oscillations in the probe. **G** Tibial bending applied as repeated increases and decreases without a hold phase approximated the magnitude and time course of ground reaction forces that have been recorded during walking. Bending in the direction of joint extension elicited alternating bursts of activity from the tibial campaniform sensilla even though bending in the direction of forced flexion had not occurred.



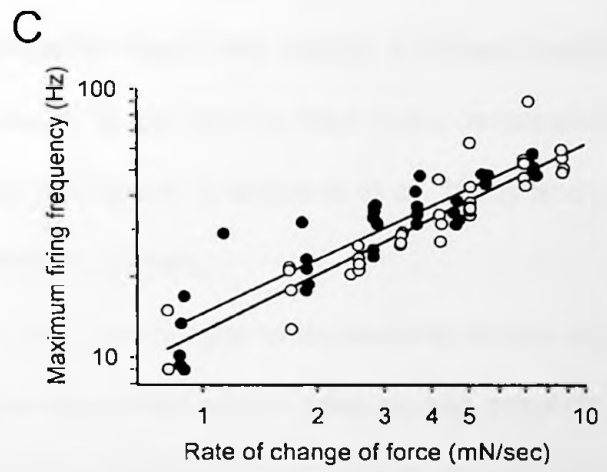
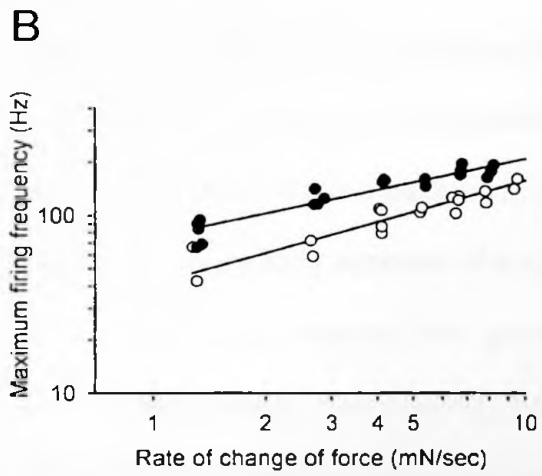
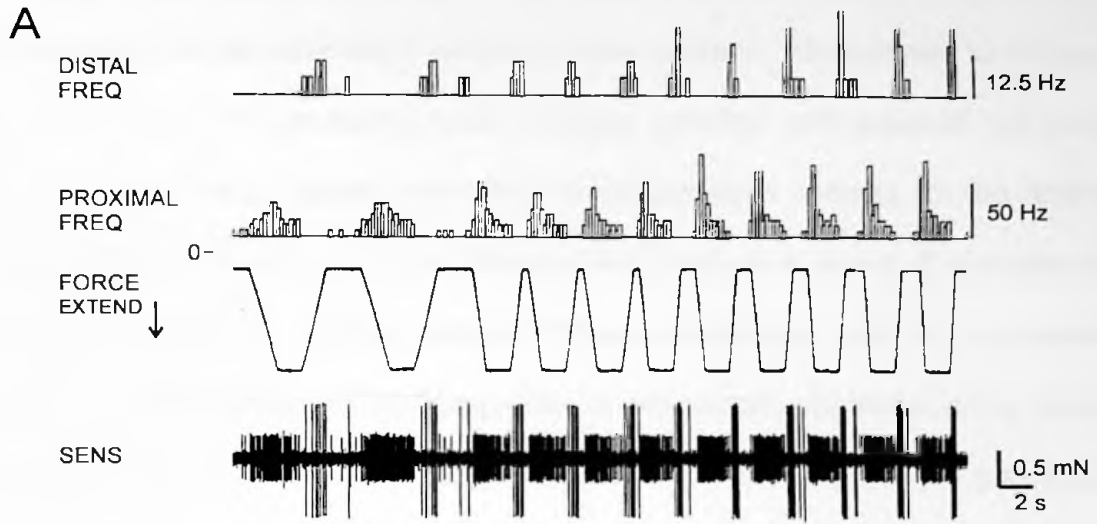
substantial hold phase, elicited reciprocal discharges in proximal and distal campaniform sensilla in the same pattern, with consistent bursts during force decrements (Fig. 2G).

Do these discharges simply signal the occurrence of declining force levels or do they also encode the rate of force decrease? To address this question, we applied ramp and hold stimuli at varying rates of rise and decline (Fig. 3). Firing of proximal sensilla during decreases from forced flexions consisted of intense bursts of activity in the range of 50 to 200 Hz that showed strong correlation with the velocity of force changes. In contrast, distal sensilla discharged at much lower frequencies with fewer action potentials to ramps declining from forced extensions. At low amplitudes of bending, the distal sensilla fired single spikes and could thus only indicate the occurrence of force decreases. Change in bending levels of higher magnitude, however, could elicit bursts that showed distinct modulation of firing frequency according to the rate of force decline (Fig. 3A). To compare the sensitivities to rate of change in applied force, we plotted the maximum firing frequencies during phasic discharges of single campaniform sensilla. These plots show a dependence upon force velocity as a power function in both proximal and distal sensilla during both increasing and decreasing ramps (Figs. 3B, C and legends). However, similar results were also seen in other experiments (N=3) in which forces were applied in both directions and sensitivities to rate of change were observed in all experiments in which sensilla discharged with multiple spikes. Further experiments are necessary to assess these different sensitivities quantitatively and to characterize the effect of force amplitude on afferent firing.

DISCUSSION

The present study has shown that the tibial group of campaniform sensilla can actively

Figure 3: Encoding of rate of force increase and decrease. **A** Bending forces were applied as ramps of varying rate but identical amplitude. A series of force extensions (down on FORCE trace) elicits discharges in proximal and distal sensilla during respective force increases and decreases that varied in frequency with the ramp rate. **B** Plot of maximum discharge frequencies of a proximal sensilla during the increasing ramp phase of forced extension (filled circles, $r^2 = 0.935$, slope = 7.6, y-intercept = 109.3, $p < 0.01$) and the decreasing phase of forced flexion (hollow circles, $r^2 = 0.964$, slope = 7.9, y-intercept = 68.6, $p < 0.01$) during bending tests in a single preparation. Both discharges to increasing and decreasing forces showed rate sensitivities with similar slopes. **C** Plot as in B for a distal sensillum for tests of rate sensitivities to force increases (filled circles, $r^2 = 0.782$, slope = 5.68, y-intercept = 12.8, $p < 0.01$) and decreases (hollow circles, $r^2 = 0.893$, slope = 3.85, y-intercept = 18.1, $p < 0.01$).



signal both increases and decreases in bending forces. These findings are important in understanding how forces are encoded by these receptors and in evaluating their function as inputs to the cockroach walking control system. Responses to decreasing levels of force have not previously been explicitly reported and studied, but they are evident in some earlier published recordings of campaniform sensilla in the cockroach (Spinola and Chapman 1975; Zill and Moran 1981) and stick insect (Delcomyn 1991). However, the potential information content of these discharges was not systematically examined. Similar responses to declining levels of sinusoidally applied bending have also recently been reported for the locust tibial campaniform sensilla (Newland and Emptage 1996), but the individual receptors and the orientation of their cuticular caps were not identified. It was, therefore, unclear whether those responses implied a bidirectionality in unit discharges. Discharges to decreasing levels of leg loading have been demonstrated in groups of cuticular force receptors in other arthropods (Marchand et al. 1995) and may be present in the walking systems of a number of animals.

The mechanisms underlying the generation of discharges to decreasing forces are, at present, undetermined. Our finding, that the orientation of the cuticular cap predicts the directionality of the tibial sensilla to declining forces, suggests the parsimonious hypothesis that the cap provides a final common mechanism of transduction for both force increases and decreases. The generation of these diverse responses could then depend upon the specific temporal and spatial distribution of strains within the exoskeleton, which can also show viscoelastic properties that could contribute to responses to decreasing forces (Blickhan and Barth 1985). The present findings are in clear contrast to the bidirectional responses obtained from campaniform sensilla of dipteran wings (Dickinson 1992) which

differ from leg sensilla in the shape of the cuticular cap and sensory dendrite. Thus, the morphological and mechanical properties responsible for the discharges to declining forces remain a subject for further investigation.

What are the potential advantages in actively signaling decreases in load in a walking system? First, the information that forces are declining is prerequisite for the initiation of leg lifting in swing (Whelan et al. 1995; Bässler 1987) and also enhances the placement of other legs in support (Bässler et al. 1991). These functions could be accomplished by monitoring the declining frequency of a receptor that simply encodes the level of load. However, such a signal could be compromised by processes such as adaptation or hysteresis, which are present in campaniform sensilla and common in many sensory systems (Zill and Moran 1981; French 1992). Furthermore, the force that a leg exerts must drop substantially when the foot or point of contact slips on the substrate. The system would therefore be providing an active signal to allow for the initiation of the necessary rapid compensatory reactions (Jacobs and Macpherson 1996). The selective responses of the tibial sensilla to both decreases and increases in load provide a mechanism for anticipating the need for further support and for initiating rapid responses to maintain stable postures. Experiments are planned to test these hypotheses in the cockroach. However, other biological and control systems may utilize signals of force decrements to similar advantage.

CHAPTER 2

ENCODING OF FORCES BY COCKROACH TIBIAL CAMPANIFORM SENSILLA: IMPLICATIONS IN DYNAMIC CONTROL OF POSTURE AND LOCOMOTION

SUMMARY

Forces exerted by a leg in support and propulsion can vary considerably when animals stand upon or traverse irregular terrains. We characterized the responses of the cockroach tibial campaniform sensilla, mechanoreceptors which encode force via strains produced in the exoskeleton, by applying forces to the leg at controlled magnitudes and rates. We also examined how sensory responses are altered in the presence of different levels of static load. All receptors exhibit phasico-tonic discharges that reflect the level and rate of force application. Our studies show that: 1) tonic discharges of sensilla can signal the level of force, but accurate encoding of static loads may be affected by substantial receptor adaptation and hysteresis; 2) the absolute tonic sensitivities of receptors decrease when incremental forces are applied at different initial load levels; 3) phasic discharges of sensilla accurately encode the rate of force application; and 4) sensitivities to changing rates of force are strictly preserved in the presence of static loads. These findings imply that discharges of the sensilla are particularly tuned to the rate of change of force at all levels of leg loading. This information could be utilized to adapt posture and walking to varying terrains and unexpected perturbations.

INTRODUCTION

Inputs from sense organs that detect mechanical forces acting upon the legs are now considered essential components in the control of posture and locomotion, in both vertebrate and invertebrate animals (vertebrates, Pearson et al. 1992; Dietz 1998; Pearson et al. 1998; invertebrates, Ridgel et al. 1999; review, Duysens et al. 2000). These types of receptors can indicate the extent of loading and signal whether the levels of force exerted by limb muscles are adequate to provide support during standing and walking. For example, the magnitude of loading during the start of the stance phase of locomotion is signaled by receptors that reflexively adjust the activities of extensor muscles to generate forces of appropriate amplitudes to support the animal's weight (Pearson and Collins 1993; Bässler et al. 1991). At the end of stance, loading must be decreased to critical levels for the initiation of leg lifting in swing (Pearson et al. 1992; Pearson et al. 1998; Whelan et al. 1995) and for normal coordination of leg movements (Bässler 1987).

Recent work has also stressed that the regulation of force dynamics (dF/dt) may play a key role in determining responses to perturbations of posture and locomotion (Jacobs and Macpherson 1996; Macpherson 1988a,b), as well as the adaptation of walking patterns to irregular or unstable terrains (Mcilroy and Maki 1994). During postural perturbations in subjects standing upon a moveable platform, the specific activities of groups of limb muscles were strongly correlated with the direction of force application and these patterns of activation may be organized according to the resultant force vectors (Jacobs and Macpherson 1996; Horak and Macpherson 1996; Macpherson 1988a,b). These responses were adjusted to the magnitude and rate of the platform displacement and

occurred at short latencies (Burleigh and Horak 1996), implying that they are tuned to these variables by receptors that monitor force dynamics. Furthermore, perturbations of larger magnitudes and rates can elicit stepping responses (Burleigh and Horak 1996; Mcilroy and Maki 1994; Burleigh et al. 1994; Mcilroy and Maki 1993; Maki et al. 1993), in which a leg is lifted and repositioned so that the center of gravity or pressure is within the base of support. Those studies imply that the nervous system is able to rapidly detect and process information about changes in force to adopt appropriate compensatory strategies and to prevent falling.

However, the specific types of sense organs that encode these diverse forces and the mechanism by which they detect dynamic parameters remain somewhat controversial. In vertebrates, forces acting upon the head and body are detected directly by two systems, the vestibular apparatus (Mittlestadt 1998; Inglis and Macpherson 1995; Macpherson and Inglis 1993) and proprioceptive afferents (Pearson et al. 1992; Pearson et al. 1998). Inputs from both types of receptors have been shown to be integrated into reactions to postural perturbations during standing and walking. Forces acting on the limbs are now thought to be monitored by Golgi tendon organs (Whelan et al. 1995; Hiebert et al. 1995; Heibert et al. 1994) and some tendon organs have been shown to be able to encode the rate of applied force (Davies et al. 1995; Jami 1992; Fukami and Wilkinson 1977; Goslow et al. 1973). However, the specific way force information provided by Golgi tendon organs is incorporated into postural perturbations remains undetermined.

In contrast, many arthropods lack a vestibular system and forces are thought to be detected entirely by proprioceptive sense organs of the limbs (cuticular stress detectors,

Marchand et al. 1995; campaniform sensilla, Pringle 1938a; lyriform organs, Blickhan and Barth 1985; muscle receptor organs, Tryba and Hartman 1997; overview, Zill and Seyfarth 1996). In insects, campaniform sensilla detect forces on the legs as strains in the exoskeleton (Pringle 1938b; Hofmann and Bässler 1982, 1986; Delcomyn 1991; Schmitz 1993; Zill and Seyfarth 1996). The strains are monitored via small cuticular caps that form the attachment points for the sensory dendrites (Moran et al. 1971). The studies of Chapman and colleagues (Chapman et al. 1973, 1979; Chapman 1975; Spinola and Chapman 1975) extensively characterized the mechanisms of sensory transduction of the cockroach tibial campaniform sensilla by mechanical stimulation of the cuticular caps. However, few previous studies have examined sensory responses to forces exerted upon the leg. Zill and Moran (1981a,b) confirmed the findings of Spinola and Chapman (1975) and established that the tibial sensilla respond directionally to force applied to the leg and that afferent discharges were maximal in response to forces acting close to the plane of movement of the adjacent femoro-tibial joint. In those studies, the magnitudes of forces that were applied to the leg were not directly monitored (but only estimated) and the rate of force application was not controlled. These problems have limited subsequent attempts to model the responses and functions of campaniform sensilla (Cocatre-Zilgien and Delcomyn 1999).

The goals of the present study were: 1) to characterize the basic responses of the tibial campaniform sensilla to controlled and measured force applied to the leg and 2) to particularly examine the effects of increased loading upon receptor function. Dynamic variations that occur in the presence of static loads may be important in adapting posture and locomotion to uneven or unstable terrains, in both animals and walking

machines (Duysens et al. 2000; Nelson and Quinn 1998; Nelson et al. 1997). Our studies have shown that the receptors are effective encoders of level but that signals from the largest sensilla could be compromised by adaptation to sustained loads and hysteresis. In contrast, sensitivities to changing levels of force (dF/dt) are accurately maintained in the presence of sustained loads. These studies confirm elements of previous evaluations of the functions of campaniform sensilla in the control of posture and walking (Zill and Moran 1981a; Schmitz 1993; Zill 1993; Prochazka 1996). These types of sensitivities may be important in the walking system during adaptive locomotion.

MATERIALS AND METHODS

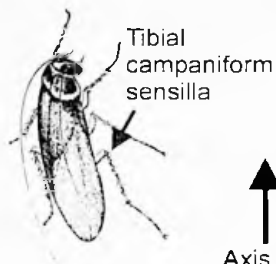
Adult male *Periplaneta americana* ($n= 24$) were anaesthetized with carbon dioxide and secured, ventral side up, to a Sylgard resin-coated block using staples made from insect pins (Fig. 4) (see methods of Ridgel et al. 1999). Under a dissecting scope, the nerves that provide sensory and motor innervation to the right metathoracic leg (Nijenhuis and Dresden 1956) were cut under the cuticle near the metathoracic ganglion. After clotting of the wound occurred, the animals were returned to a plastic jar with a water source for 20 minutes, then re-anaesthetized and secured, dorsal side up, on the resin block.

Extracellular recordings from the tibial campaniform sensilla (Figs. 1A,B; Group 6 of Pringle 1938b) were obtained by placing 50 μm silver wires near nerves 5 or 5r8 (Nijenhuis and Dresden 1956) through holes made in the cuticle of the femur (Fig. 4C). These electrodes monitored activities of the largest sensilla of both subgroups (proximal MA, MP and distal DA of Spinola and Chapman 1975; Fig. 4D) as their axons are

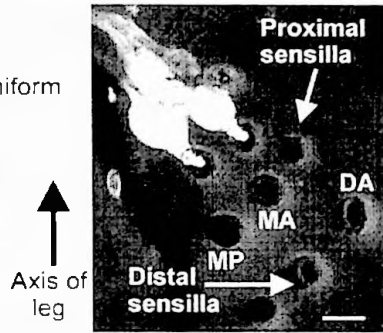
Figure 4: Experimental setup and identification of unit discharges to applied forces. **A** The tibial campaniform sensilla are located on the proximal end of the tibial segment of the leg (drawing after Schnorbus 1971). **B** Confocal projection image of tibial campaniform sensilla. The tibial campaniform sensilla consist of two subgroups, proximal and distal sensilla, which have cuticular caps with mutually perpendicular orientations. The dendrites of two proximal sensilla, stained with Dil, are shown inserted into their respective cuticular caps. (calibrate = 20 microns). **C** Diagram of experimental setup. Animals were restrained on a resin block and the femoro-tibial joint was stabilized by gluing it to a small pin (Pin). Sensory recordings (Sens) were obtained from wire electrodes placed adjacent to nerve branches in the femur. Forces were applied to the tibia via a probe (Force probe) mounted to a piezoelectric crystal. Ramp and hold waveforms (Ramp generator) were applied to the crystal (PE amplifier) and forces were monitored through strain gauges on the probe (SG amplifier). Individual sensilla were identified by indenting their caps with a fine wire (Indent caps). All data were recorded on tape (Tape recorder). **Di-iii** Identification of units by cap stimulation/ablation. Force applied to the tibia in the direction of forced extension (i) excited a small amplitude unit (proximal sensilla) and release of force excited a large amplitude unit (distal sensilla) (Ridgel et al. 1999). Indentation of an individual proximal sensilla cap elicited an intense discharge that matched the amplitude of the response recorded in Di (data not shown). Ablation of this proximal sensilla cap (ii) produced an injury discharge of high frequency followed by no further activity. Cap ablation led to an elimination of discharges to increased force (iii). However, discharges from the large amplitude unit (distal sensilla) upon release of force could still be recorded. **E** The force probe was calibrated with small weights and was equally

sensitive to forces in both directions (filled circles = direction of force extension, $r^2 = 0.979$; open circles = direction of force flexion, $r^2 = 0.996$).

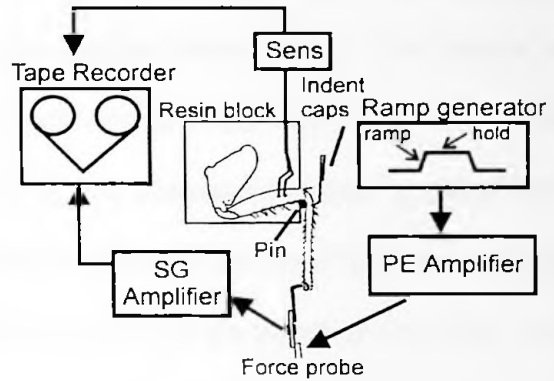
A



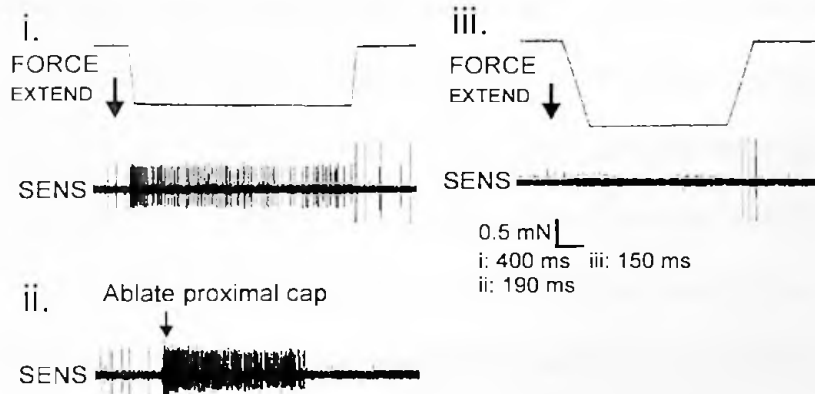
B



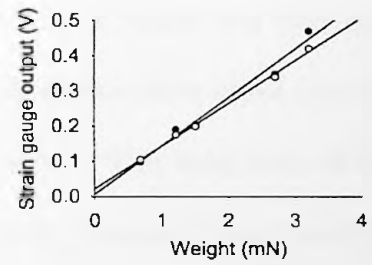
C



D



E



contained in the same nerve branches (Schnorbus 1971; Zill et al. 1980). The smaller sensilla have not been systematically studied, presumably due to the fact that they have smaller axonal diameters and are much more difficult to record using extracellular techniques. The wires, which were bared of insulation near their tips, were secured using cyanoacrylate glue and tied to the leg with copper wire (#40). The tarsus was then severed and the spines on the tibia and distal femur were cut. The femoro-tibial joint was immobilized in either a partially flexed or completely extended position with a small pin that was placed adjacent to the joint and secured to the joint and the resin with glue. This arrangement allowed for unimpeded bending of the shaft of the tibia, which was restrained only at its proximal end. Forces were imposed upon the distal end of the tibia, in the plane of the femoro-tibial joint, using a probe that was displaced by a piezo-electric crystal (Fig. 4C, Force probe). The probe consisted of a metal rod that was waxed to a piece of shim steel. Two strain gauges were glued to the shim steel (one on each side) and linked in a Wheatstone half-bridge configuration. The free end of the metal rod was pressed against either the inner (ventral) or outer (dorsal) surface of the distal tibia. Ramp voltages were then applied to the piezo-electric crystal. As the joint was immobilized, these stimuli effectively bent the tibia in the direction of extension (which we term FORCED EXTENSION) or flexion (termed FORCED FLEXION). The resultant forces were monitored via a custom built strain gauge amplifier. In other experiments, progressively increasing stimuli were applied to the leg through a hydraulic micromanipulator (Narishige, Japan) which held the force probe and crystal. At the end of an experiment, individual campaniform sensilla were identified by indenting their cuticular caps with a fine etched tungsten wire attached to another piezo-electric crystal,

as previously described (Ridgel et al. 1999) (Fig. 4C, D). All stimuli and sensory recordings were amplified and stored on digital audio tape (DAT).

Experiments were transcribed to computer using a CED 1401 analog to digital converter and Spike2 3.0 software (Cambridge Electronic Design, England). Data were analyzed in Spike2 3.0. Force measurements were calibrated by placing weights made of solder wire on the end of the metal rod of the probe (Fig. 4E). All plots were obtained using Sigma Plot 4.0 (SPSS, Inc) and statistics were calculated using Sigma Stat 1.0 (SPSS, Inc) and StatView 5.0 (SAS Institute).

Receptor cells (Fig. 4B) were stained with Dil (after methods of Zill et al. 1993) and imaged with a Bio-Rad confocal microscope (Larsen et al. 1997).

RESULTS

Identification of Sensory Units

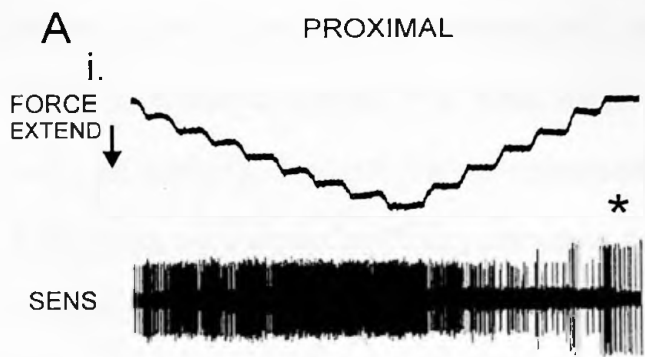
Vigorous discharges to bending of the tibia were recorded from nerves in the femur. Typically, the activities of one or two units of small amplitude were recorded upon forced extension (Fig. 4Di) while a single large unit was recorded during bending which forced the tibia in the direction of flexion (Fig. 5B) (Chapman 1975, Schnorbus 1971). A number of controls were performed to confirm the identities of these units as individual proximal or distal tibial campaniform sensilla. First, we applied force to the end of the tibia, then shifted the point of force application to a point approximately halfway along the length of the tibia. This control confirmed that the discharges were elicited to tibial bending and were not dependent upon the point of contact of the probe, which could inadvertently stimulate the large tibial tactile spines. Second, at the end of an experiment we mechanically stimulated the caps of Group 6 sensilla individually with a

fine etched tungsten wire probe (distal sensilla $n= 12$, proximal sensilla $n= 11$) (Ridgel et al. 1999). Cap indentation produced phasico-tonic discharges that could be quite intense and often closely matched the amplitude of the responses recorded to tibial bending. However, the extracellular recorded amplitude could be affected by prolonged bending or by the rapid displacements that were used to record responses to stimuli of different velocities. We, therefore, compared spike amplitudes on the last series of tests recorded prior to cap indentation ($n= 13$). In 11 preparations, we also ablated the caps of individual sensilla, most often by forcefully depressing the probe used for cap stimulation (Fig. 4Dii). In experiments in which only single proximal or distal sensilla were recorded, cap ablation led to the complete elimination of discharges to forced extension or flexion (Fig. 4Diii). These ablations also could selectively eliminate the phasic discharges of units to decreasing force levels and confirmed that these responses were derived from campaniform sensilla (Ridgel et al. 1999).

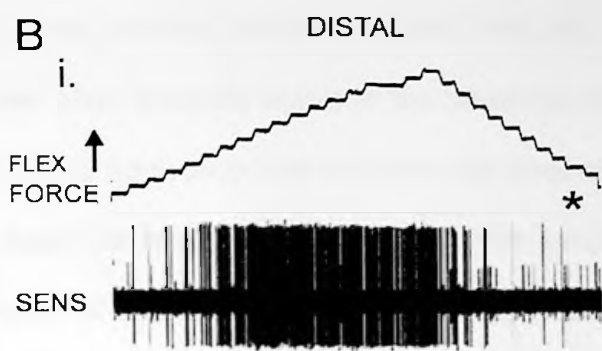
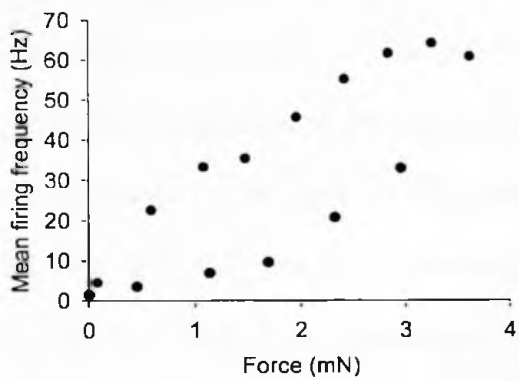
Responses to 'staircase' stimuli

We first characterized the responses of Group 6 sensilla to different amplitudes of force by progressively bending the tibia with the femoro-tibial joint immobilized. These 'staircase' stimuli were applied to the distal tibia by manually displacing the horizontal-axis control knob of the micromanipulator which held the force probe. Figure 5 shows responses recorded during progressively increasing, and then decreasing, force which bent the tibia first in the direction of joint extension (Fig. 5Ai) and then toward joint flexion (Fig. 5Bi). Responses of individual sensilla to increasing staircase stimuli were strictly directional and correlated with the orientation of the receptor's cuticular cap. In all cases, the proximal sensilla fired to forced extension while the distal sensilla fired to

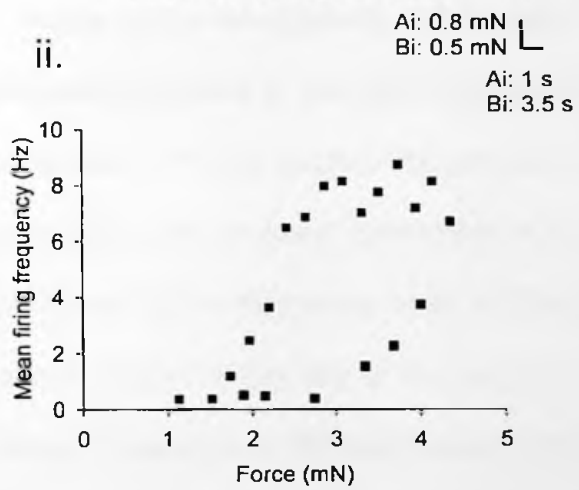
Figure 5: Response to increasing and decreasing forces applied as 'staircase' stimuli. Forces applied as progressive staircases (through the micromanipulator that held the force probe) elicited tonic discharges in tibial sensilla. Proximal sensilla (**Ai**) were excited upon force extension (down on force trace) while distal sensilla (**Bi**) responded to force flexion (up on force trace). The graphs plot the mean firing frequency of the receptors (**Aii**, proximal; **Bii**, distal) versus the level of sustained force for the discharges shown in Ai and Bi. The sensilla show linear increases in discharge rate that encode the force level but saturate at higher levels of applied force. Tonic firing exhibits substantial hysteresis when force levels were decreased (arrows signify increasing and decreasing forces). In addition, force decreases were also accompanied by discharges of sensilla of the opposite cap orientation (asterisks in Ai and Bi).



ii.



ii.



forced flexion. Figures 5Aii and 5Bii plot the mean discharge frequency in the entire hold phase at each level during increases and decreases in force application (see Figs. 5Ai, 5Bi). The proximal sensilla (Fig. 5Aii), which were often tonically active in the absence of imposed bending, showed graded increments in firing frequency that reflected the level of force but response sensitivities decreased at levels above 3 mN. In contrast, the distal sensilla (Fig. 5Bii) fired phasically at low levels of applied force and only exhibited sustained discharges at higher force amplitudes. These responses showed considerable saturation at levels above about 3 mN, and subsequent increases in the level of applied force did not result in higher afferent firing frequencies. When applied forces were decreased from maximal amplitudes, sensilla discharged at much lower rates relative to that seen during force increases. This hysteresis, shown in the frequency plots in Figs. 5Aii and 5Bii, resulted in a 58-85 percent decrease in afferent firing rate in the range of 1.5-2.5 mN for proximal sensilla and a 74-95 percent decrease in afferent firing in the range of 2-4 mN for distal sensilla. Similar hysteresis has previously been reported for the trochanteral campaniform sensilla in the stick insect (Hofmann and Bässler 1986) and in a number of other mechanoreceptors (e.g. chordotonal organ, Zill and Jepson-Innes 1988).

We also consistently observed activity during the decreasing phase of the 'staircase' that was derived from the campaniform sensillum of the opposite cap orientation. These types of discharges were phasic but occurred during decreases from forces applied as forced extensions and forced flexions, even though active bending in the opposite direction had not occurred. For example, the discharge of a distal sensillum occurs at the end of the staircase sequence of forced extension (Fig. 5Ai), even though

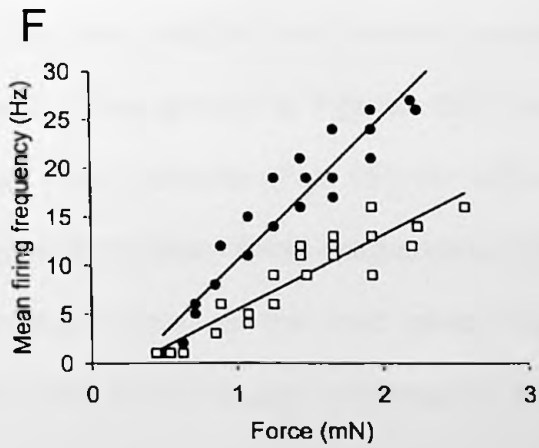
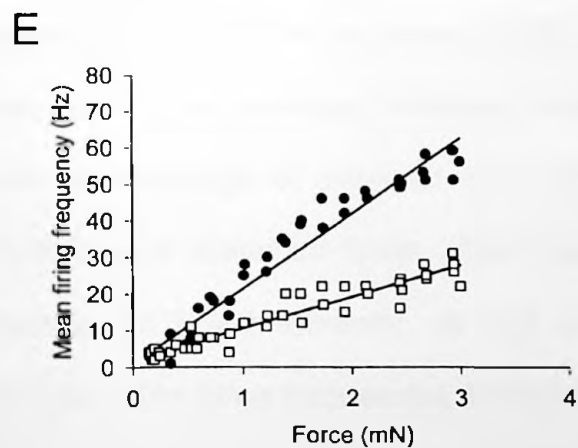
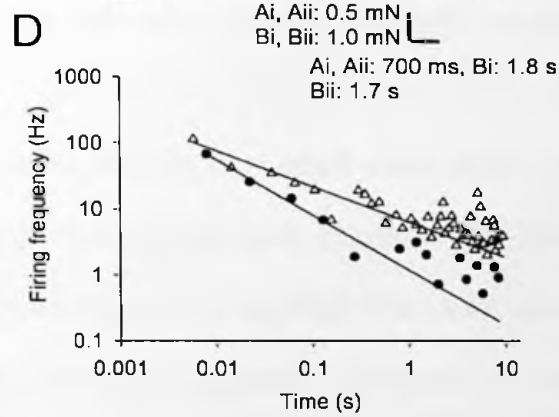
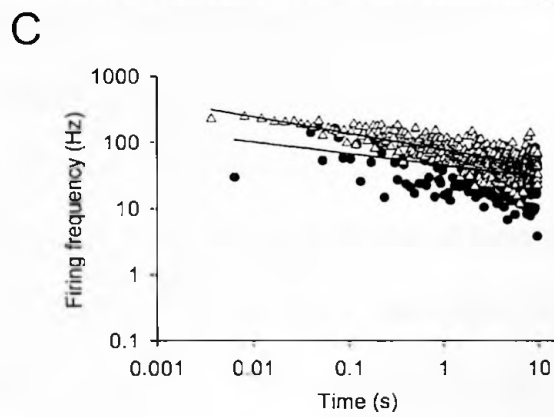
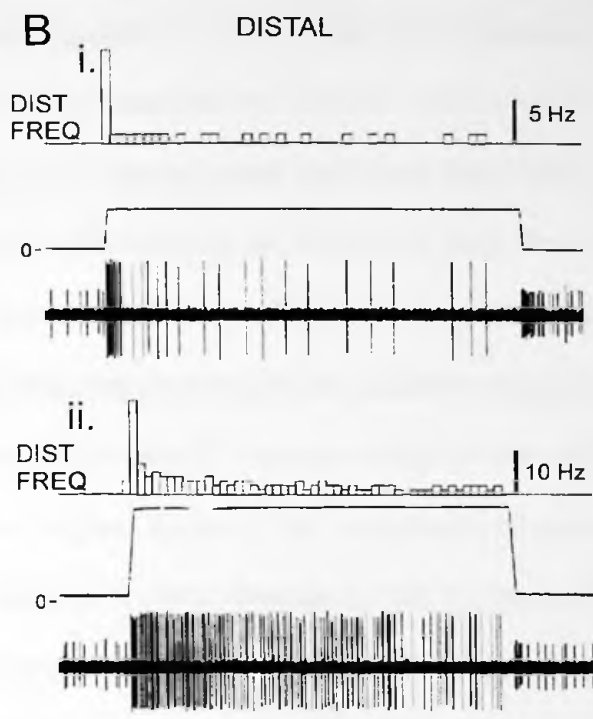
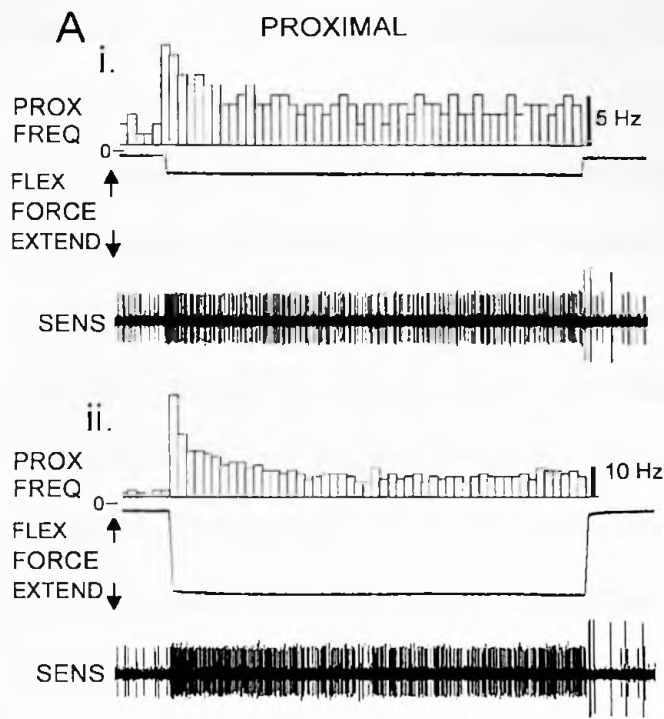
the level of applied force had not reached zero and no bending had been imposed in the direction of forced flexion (Ridgel et al. 1999). These responses were also observed in the tests using ramp and hold stimuli (see Figs. 6-9). These findings support the idea that the tibial campaniform sensilla maintain their individual directionality but that firing can occur both in the preferred direction and from release of force applied in the opposite direction.

Responses to ramp and hold stimuli

We studied responses to varying levels of force more extensively by using ramp and hold stimuli that had constant rates of force application but variable amplitude. All campaniform sensilla exhibited phasico-tonic discharges that had high firing frequencies during the ramp phase which adapted to lower levels during the hold phase (Spinola and Chapman 1975, Zill and Moran 1981b). However, the proximal and distal receptors differed in their sensitivities and rates of adaptation. Figure 6 shows the discharges of proximal (Fig. 6Ai,ii) and distal sensilla (Fig. 6Bi,ii) to ramps that reached different levels of sustained force applied to the distal tibia (Figs. 6A, B are from two different preparations). The firing frequencies of the proximal receptors to forced extension were consistently higher than those of distal receptors to forced flexion, at all levels of force application that were tested. Furthermore, the initial phasic discharge was more prolonged in the proximal sensilla than in the distal sensilla. At low levels of force, activity of the distal receptors often adapted completely as was seen in responses to 'staircase' stimuli.

These differences in the rate of adaptation and their dependence upon the amplitude of the stimulus are evident in the graphs of sensory discharge shown in Figs. 6C-D.

Figure 6: Responses to ramp and hold stimuli and receptor adaptation. **A-B** Forces were applied to the tibia via voltages delivered to the piezoelectric crystal of the probe. Proximal sensilla (**A**) exhibit phasico-tonic discharges to forces applied as ramp and hold stimuli in the direction of extension (down arrow in Ai,ii). Distal sensilla (**B**) show similar responses to force flexion (up arrow in Bi,ii). The histogram above the recordings plots the firing frequency in bins of 50 msec. Tonic discharges of proximal receptors occur at higher sustained frequencies and adapt less rapidly than the distal receptors (force level: **Ai** 0.6 mN, **Aii** 1.9 mN, **Bi** 1.3 mN, **Bii** 3.0 mN). **C-D** Sensillum adaptation during the hold phase. Adaptation rates were evaluated by plotting the afferent discharges during the hold phase of the stimulus, beginning at the cessation of the rising phase of the ramp. The instantaneous firing frequencies of a proximal (**C**) and distal (**D**) sensillum were plotted during a 9 sec stimulus. The proximal receptors adapt slowly at all force levels (triangles = 2.2 mN, circles = 1.2 mN; rate: 15.8 mN/sec). In contrast, firing of the distal receptors adapted rapidly and was only more sustained at higher levels (triangles = 2.0 mN, circles = 1.1 mN; rate = 15.9 mN/sec). **E-F** Encoding of static loads. Ramp and hold stimuli were applied at constant rates of rise but progressively increasing amplitudes. The mean tonic firing frequencies during second 1-2 (circles) and the last second (squares) of the hold phase are plotted for series of stimuli in two different preparations (**E** proximal sensilla; **F** distal sensilla). Afferent discharges encode the static level of force during both intervals. The firing frequencies of the proximal receptors to forced extension are consistently higher than those of distal receptors to forced flexion. (E circles, slope = 20.5 $r^2 = 0.959$; squares, slope = 8.6 $r^2 = 0.911$; F circles, slope = 14.8, $r^2 = 0.926$; squares, slope = 7.7, $r^2 = 0.842$)



Figs. 6C and 6D plot the instantaneous firing frequency during the hold phase at comparable levels of force (amplitude 1.1-2.2 mN) (Chapman and Smith 1963; French and Torkelli 1994). These discharges are plotted on a log-log scale and have been fitted to the exponential equation $y = At^{-k}$ according to the method of Chapman and Smith (1963). The power coefficients (k) in this example are 0.17 and 0.25 for proximal sensilla and 0.84 and 0.86 for distal sensilla. The discharge frequencies of the proximal sensilla adapt slowly at both levels while firing rate of the distal sensilla adapts rapidly to low and moderate force levels and is more sustained at higher forces. The discharges of both types of receptors also showed considerable variation in firing frequency late in the hold phase (Fig. 6C,D). However, at these levels of imposed force, the firing of both types of sensilla during the last second of the hold phase still reflected the amplitude of the stimulus.

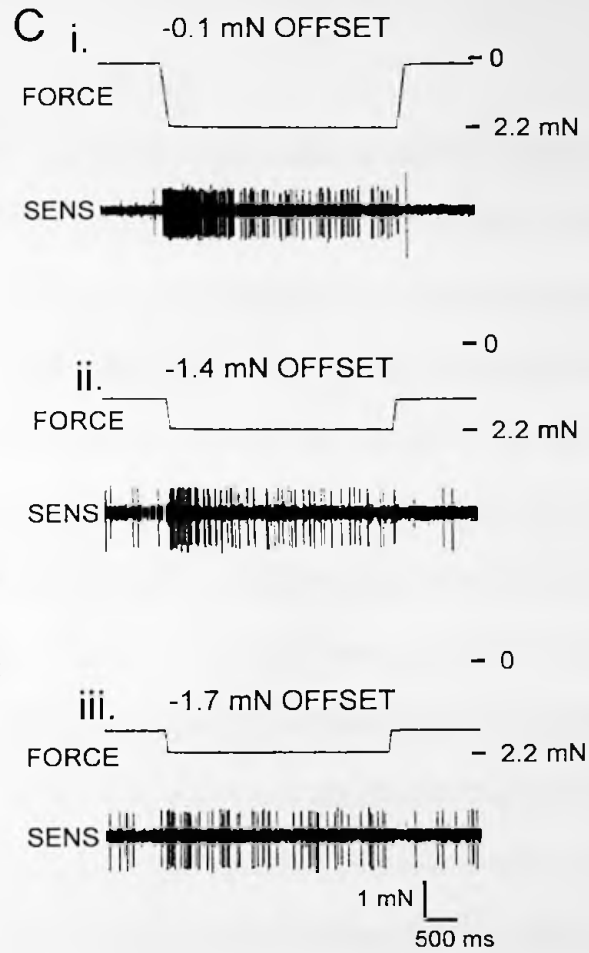
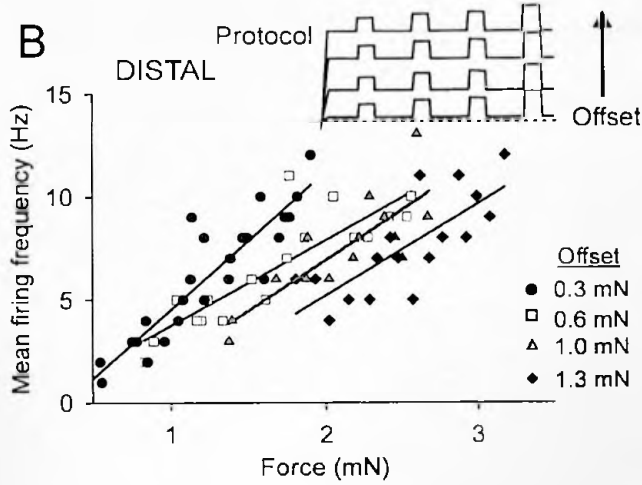
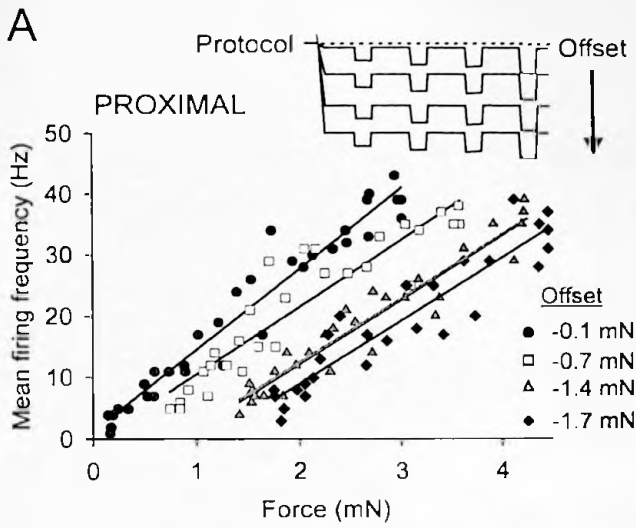
In order to assess the abilities of the campaniform sensilla to encode static loads, we imposed ramp and hold stimuli of long duration (9-10 seconds) and increased the hold level in successive tests. Each ramp and hold stimulus was applied from the same baseline with a 1-5 second interval between force applications. Because of the hysteresis exhibited by the receptors (Fig. 5), our data analysis was limited to stimuli presented at successively increasing force levels. The graphs in Figures 6E,F show rates of discharge of proximal (Fig. 6E) and distal sensilla (Fig. 6F) for different amplitudes of sustained force. Each graph plots the mean firing frequencies during seconds 1-2 (filled symbols), as well as the last second of the hold phase (open symbols). The firing frequencies during both periods systematically increased as larger

forces were applied, and similar results were obtained in other preparations. The tibial campaniform sensilla linearly encoded the levels of sustained forces over these ranges.

Effects of pre-loading upon amplitude sensitivity

We studied the effects of application of pre-existing loads upon encoding of force level in similar tests, but initiated them as series at different static offsets. In these experiments, force applied to the tibia was first increased through the micromanipulator and held for 60 seconds. Ramp and hold stimuli, that had constant rates of rise and decline but variable amplitude, were then initiated as a series through voltages applied to the PE crystal of the force probe (see inset in Fig. 7). To quantify these results, we plotted the mean firing frequencies of the receptors during the interval from second 1-2 of the hold phase at each amplitude. We found this interval to be a more reliable indicator of afferent responses due to the variability in the discharge frequency seen after more extensive receptor adaptation (discussed above). Fig. 7A contains a plot of the tonic firing frequency of proximal receptors vs. the absolute force magnitude during series that were initiated at offset levels of -0.1, -0.7, -1.4 and -1.7 mN (force extension). Fig. 7B contains a similar plot of the discharge of distal sensilla at offsets of 0.3, 0.6, 1.0 and 1.3 mN (force flexion). The effects of prior loading were considerable and reflected a decrease in the absolute tonic sensitivity of the receptors (Fig. 7C). For example, 2.2 mN of force applied to the leg from a baseline level of 0.1 mN resulted in a discharge of 28 Hz. When the same level of force was applied after adaptation to a baseline of 1.7 mN, the resultant discharge was only 13 Hz, representing a 53% decrease in firing frequency. These findings indicate that the tonic discharges of campaniform sensilla at

Figure 7: Receptors encode levels of increasing force but change in absolute sensitivity at different static loads. The effects of response adaptation to pre-existing loads were studied at different offset levels. In each series, the offset was imposed through the micromanipulator one minute prior to the application of ramp and hold stimuli, similar to the tests in Fig. 6 (see protocol). **A-B** Mean firing frequencies during second 1-2 of the hold phase are plotted for increasing values in each series. The effects of prior loading are substantial in both proximal (**A**) and distal (**B**) sensilla and reflected a decrease in the absolute tonic sensitivity of the receptors. (**A** circle, slope = 13.0, $r^2 = 0.931$; square, slope = 10.7, $r^2 = 0.806$; triangle, slope = 10.7, $r^2 = 0.886$; diamonds, slope = 10.7, $r^2 = 0.859$; **B** circle, slope = 6.7, $r^2 = 0.836$; squares, slope = 5.2, $r^2 = 0.825$; triangles, slope = 5.8, $r^2 = 0.673$; diamonds, slope = 4.9, $r^2 = 0.632$). **C** Effect of prior load on discharge of a proximal sensillum. Sample recordings from the series plotted in A showing application of 2.2 mN force from different baseline levels. The discharge frequency is highest from a baseline of -0.1 mN (I) and is reduced from baselines of -1.4 mN (ii) and -1.7 mN (iii).

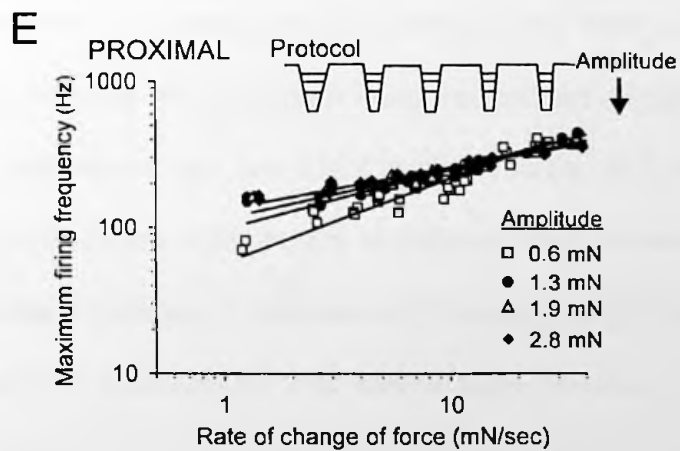
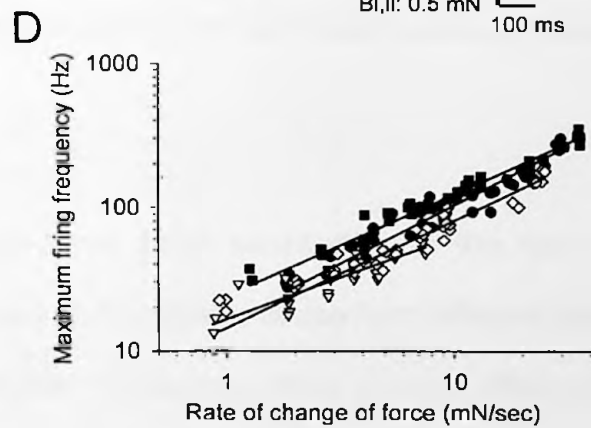
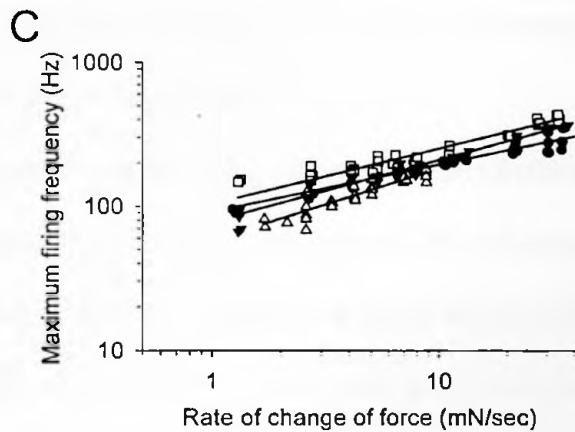
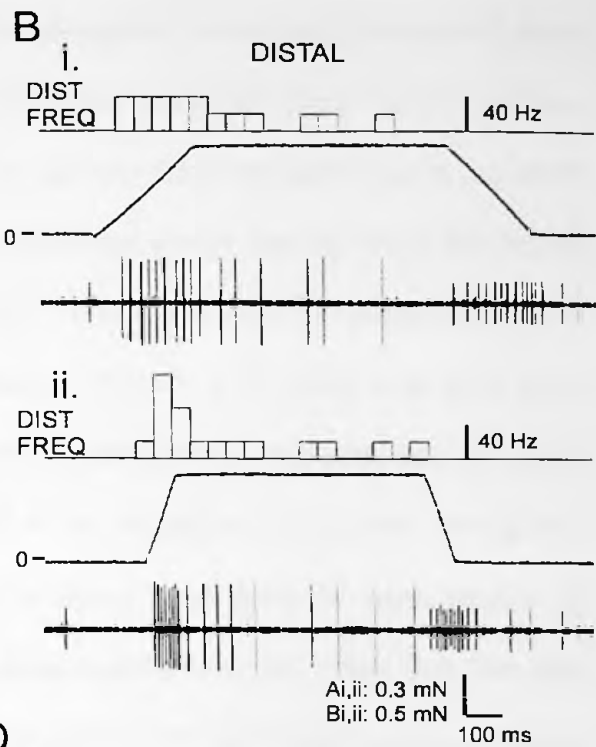
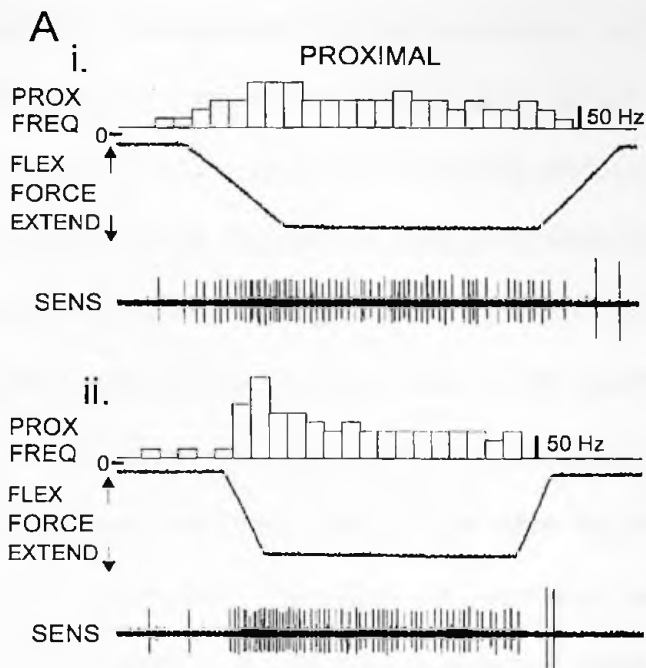


a given level of force application is not invariant, but depends upon the extent of prior application of load.

Response of sensilla to changing rates of force application

To characterize the sensitivities of the tibial campaniform sensilla to different rates of force application (dF/dt), we applied ramp and hold stimuli in which the rate of the rising and falling phase was varied ($n = 33$ distal, $n = 21$ proximal sensilla). In these tests, the stimulus amplitude was held constant so that the increase in rate resulted in a decrease in ramp duration. The discharge frequencies of all sensilla showed a strong dependence upon the rate of force application (Fig. 8). At low to moderate rates (1-12 mN/sec), sensilla often discharge at uniform firing frequencies during the ramp (Fig. 8A, B), but at higher velocities of stimulation the maximum afferent discharges did not occur until after the end of the rising phase (this was often observed in the proximal sensilla). We, therefore, plotted the responses of the sensilla as maximum discharge frequencies attained in the interval between the ramp onset and 100 ms after the end of the rising phase. Figures 8C and 8D plot the frequency of discharge of proximal (Fig. 8C) and distal (Fig. 8D) sensilla versus the rate of change of force in four different preparations. These data are plotted on a log-log scale (Chapman and Smith 1963) and indicate that the sensitivity to the rate of change of force is consistent among different preparations. Furthermore, the discharge frequencies of the proximal sensilla were consistently higher than the distal receptors at all rates tested. Data were again fitted to power functions using the methods of Chapman and Smith (1963). The power coefficients had a mean value of 0.41 (range = 0.33-0.51) for the proximal receptors but were consistently higher for the distal receptors (0.68, range = 0.64-0.75).

Figure 8: Encoding of rate of change of applied force. **A-B** Ramp and hold stimuli were applied at constant amplitudes but varying rates of rise and decline of the ramp phase. The initial, phasic discharges of both proximal (**Ai, ii**) and distal (**Bi,ii**) sensilla increased substantially when the rate of rise of the stimulus is increased. (**Ai** amplitude = 0.8 mN, rate = 7.0 mN/sec; **Aii** amplitude = 0.8 mN, rate = 2.8 mN/sec; **Bi** amplitude = 1.0 mN, rate = 12.7 mN/sec; **Bii** amplitude = 1.0 mN, rate = 4.1 mN/sec). **C-D** The maximum instantaneous firing frequencies in the initial discharges of proximal (**C**) and distal (**D**) sensilla are plotted versus rate of change of force from four different preparations. Data are graphed on a log-log scale and show linear increases in receptor firing frequency with increased rate of force application. The sensitivity of sensilla to the rate is consistent among preparations (see Table 1 for power coefficients) and the discharge frequencies of proximal sensilla are higher than distal sensilla at comparable rates. **E** Effects of stimulus amplitude upon encoding of force dynamics. A series of ramp and hold stimuli were applied at varying rates of rise and decline of the ramp phase. The amplitude of the stimulus was then increased and responses retested. This graph plots the maximum firing frequencies of a proximal sensillum versus the rate of change of force for tests using four stimulus amplitudes (squares = 0.6 mN, circles = 1.3 mN, triangle = 1.9 mN, diamonds = 2.8 mN). Firing frequencies of the sensillum are lower at 0.6 mN stimulus amplitude than at larger amplitudes. However, the sensillum fires similarly to all stimuli at rates above 10mN/sec. The sensitivity to rate of change of force is relatively constant above a minimum amplitude and velocity.



To further characterize the rate sensitivities of the receptors, we applied ramp and hold stimuli to the tibia similar to those used above but then increased the amplitude of the stimulus in successive series. Figure 8E plots the maximum firing frequencies of a proximal sensillum versus the rate of change of force for stimulus series applied from the same baseline levels with amplitudes of 0.6 mN, 1.3 mN, 1.9 mN and 2.8 mN. These data were fitted to exponential functions with power coefficients of 0.57, 0.40, 0.33 and 0.28 from lowest to highest amplitude. Firing frequencies of the sensillum to changing rates of force application were lower when ramps were applied at an amplitude of 0.6 mN than at the higher amplitudes. However, the sensillum fire similarly in all tests at rates above 10 mN/sec. Similar results were obtained in other experiments and may imply that the rate sensitivities of the proximal sensilla are relatively constant when force changes occur above a minimum amplitude.

Effects of pre-loading upon rate sensitivities

We also tested the effects of pre-existing loads upon sensitivities to the rate of change of force by applying ramp and hold series at varied rates of rise from different initial levels (Fig. 9). We again used the micromanipulator to increase force up to an offset and held that value for 60 seconds. The ramp and hold stimuli were then applied via the PE crystal of the probe. Fig. 9A plots the maximum firing frequencies of proximal sensilla to increasing rates of force application from four initial levels of force (-0.1 mN, -0.7 mN, -1.3 mN, and -1.5 mN) and Fig. 9B shows a similar plot of distal sensilla at three offsets (0.2 mN, 0.4 mN, and 0.6 mN). Table 1 provides a summary of the exponential functions calculated from these tests and those that were applied from normal baseline levels. In contrast to the

Figure 9: Receptors accurately signal rate of force application after adaptation to different static loads. Series of ramp and hold stimuli of variable rates of rise and decline of the ramp phase were applied at different initial offset levels. Plots of the responses of proximal (**A**) and distal (**B**) sensilla from single preparations indicate that the dynamic responses of the campaniform sensilla were largely unaltered even after substantial initial offsets (see Table 1 for power coefficients).

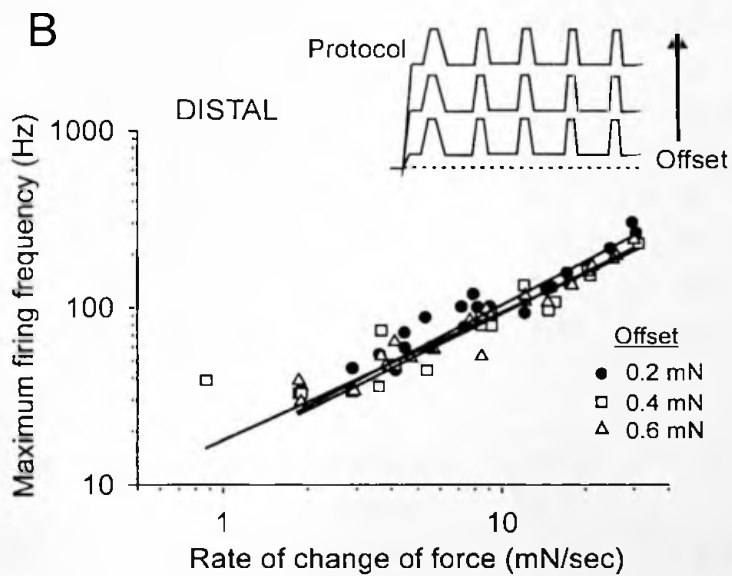
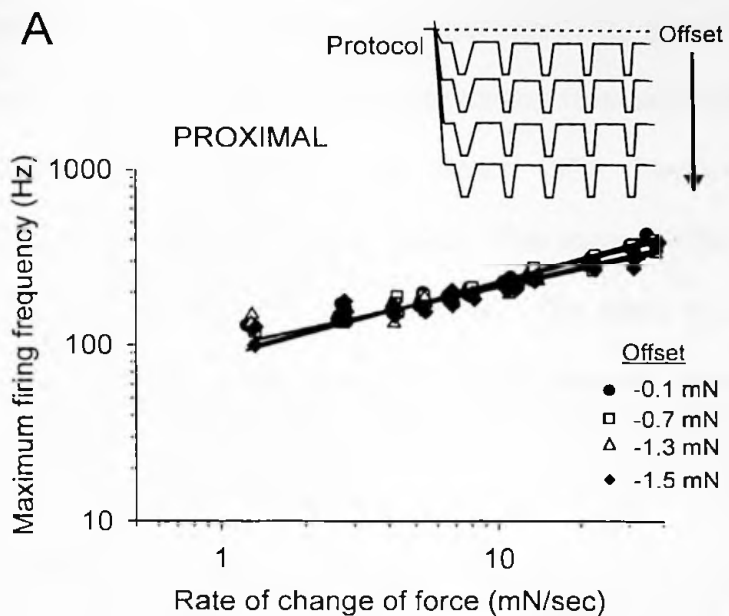


Table 1: Power and regression coefficients for tests of sensitivities to rate of change of force. Data from experiments examining rate sensitivities (Figs. 8, 9) were fitted to the equation $y=ax^k$ (Chapman and Smith 1973; Thorson and Biederman-Thorson 1974; Chapman et al 1979; French 1992). The tests in Figs. 8A,B were applied with minimum offsets in four different preparations. The tests from Figs. 9A,B were performed on single preparations with progressively increasing offsets.

Proximal

Figure	Preparation	Amplitude (mN)	Offset (mN)	a	k	R ²
8C	1	0.8	0.1	97.09	0.33	0.846
	2	1.0	0.1	78.78	0.43	0.986
	3	1.1	0.1	58.60	0.49	0.872
	4	1.4	0.1	104.3	0.38	0.951
9A	5	1.1	0.1	85.11	0.43	0.967
	5	1.1	0.7	88.97	0.41	0.969
	5	1.1	1.3	96.31	0.36	0.976
	5	1.1	1.5	97.31	0.34	0.972

Distal

Figure	Preparation	Amplitude (mN)	Offset (mN)	a	k	R ²
8D	6	0.6	0.1	15.69	0.82	0.939
	7	0.8	0.1	23.85	0.71	0.971
	8	1.0	0.1	16.62	0.58	0.809
	9	1.0	0.1	14.45	0.75	0.949
9B	10	1.0	0.2	15.69	0.82	0.939
	10	1.0	0.4	17.75	0.72	0.934
	10	1.0	0.6	15.38	0.78	0.952

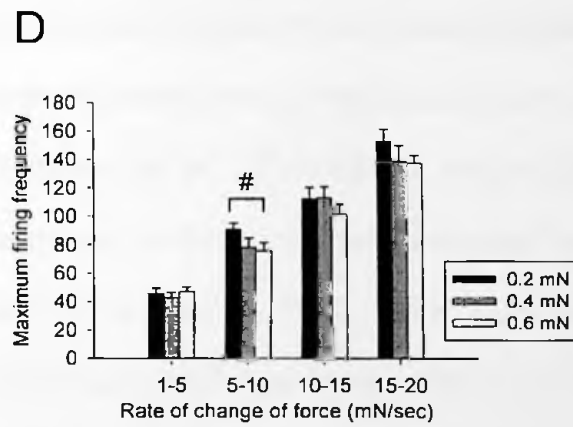
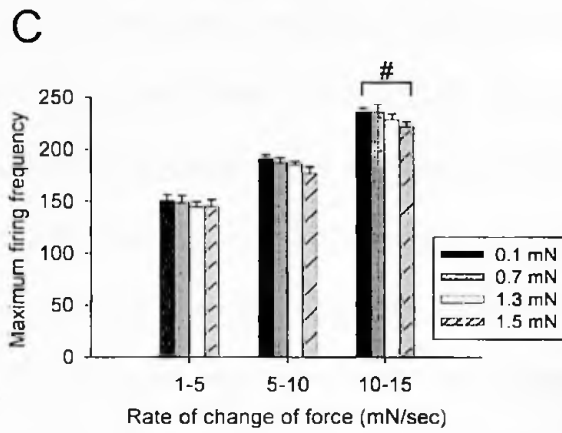
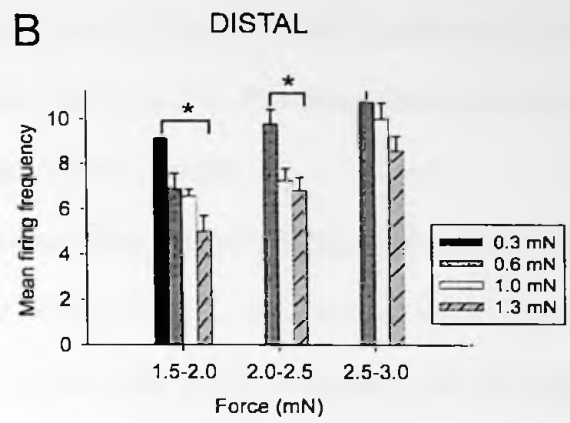
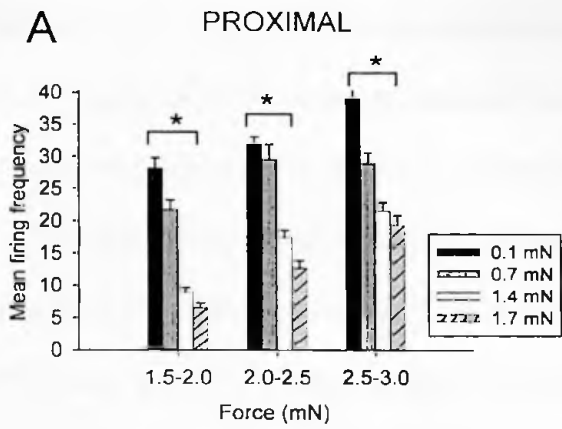
encoding of force level, the dynamic responses of the campaniform sensilla were largely unaltered even after substantial initial offsets.

Figure 10 quantitatively compares the effects of pre-loading on the tonic and dynamic sensitivities of the tibial campaniform sensilla. To compare the tonic responses, we grouped the data according to the absolute levels of force that were applied (see Fig. 7). We then calculated the mean firing frequencies of the sensilla at these levels and compared them according to the extent of pre-loading. To compare dynamic sensitivities, data were grouped according to the rate of force application. Mean afferent firing frequencies that occurred in those ranges were then calculated from the data shown in Fig. 9. An unpaired t-test was used to compare the sensilla firing frequencies at the lowest and highest offsets. Encoding of force level was significantly decreased by prior loading at nearly all levels that were compared. In contrast, sensitivities to the rate of change of force were not decreased by pre-loading, although slightly significant differences occurred in some ranges. These findings, as a whole, indicate that the detection of the rate of change of force is preserved under a wide range of loading conditions.

DISCUSSION

The accurate detection of forces applied to a leg in adaptive terrestrial locomotion requires that the system be able to monitor the levels and rates of change of those forces under a wide range of loading conditions. The present study has shown that the cockroach tibial campaniform sensilla are particularly tuned to changing forces, in that the tonic component of the discharge of the receptors can encode the level of force, but receptor sensitivities change after adaptation to static load. In contrast, the phasic component of the discharge accurately signals the rate of change of force under all

Figure 10: Summary of effects of pre-loading on afferent responses of the tibial campaniform sensilla. **A-B** Encoding of force level. Data from tests of the effects of pre-loading on tonic firing during the hold phase (Fig. 7) were grouped into ranges according to the final static level of force that was applied. The mean tonic firing frequencies of the receptors were calculated in each range. The tests were compared according to the offset load that was applied prior to reaching the final level of force. Student's t-test was used to compare the groups with the highest and lowest initial offsets. Firing frequency of the proximal sensilla (**A**) is significantly different in all three ranges of static load (*, $p < 0.01$). Encoding of force level by the distal sensilla (**B**) was significantly different in 2 of 3 ranges (*, $p < 0.01$). **C-D** Encoding of rate of change of force. Data from tests of the effects of initial offsets on sensitivities to force rate were grouped into ranges according to the rate of change of the rising phase of the ramp. Mean values of sensillum firing frequency were calculated for each range from the data in Fig. 9. The data were compared according to the static offset that had been applied. Firing frequencies of the proximal sensilla (**C**) are not significantly different in 2 of 3 ranges and only slightly different (#, $p = 0.04$) in the third range. Encoding of force rate by distal sensilla (**D**) is not significantly different in 3 of 4 ranges. These tests support the idea that sensitivity to rate of change of forces is preserved after adaptation to static loads.



loading conditions. In the following, we will discuss these results in the context of 1) findings of other studies on campaniform sensilla; and 2) the potential functions of these types of signals in the maintenance of postural stability and in the adaptation of walking.

Comparison with previous studies on leg campaniform sensilla

The response properties of campaniform sensilla of insect legs have been the subject of a number of investigations (Pringle 1938a,b; 1961; Schnorbus 1971; Spinola and Chapman 1975; Zill and Moran 1981a, b; Schmidt 1993; Newland and Emptage 1996). However, almost all previous studies that have quantitatively examined their encoding capabilities have utilized direct mechanical stimulation of the cuticular caps as a stimulus (Spinola and Chapman 1975; Chapman et al. 1973; 1979). In contrast, experiments that have characterized their responses to forces applied to the leg have typically not measured those forces (Hofmann and Bässler 1982, 1986; Delcomyn 1991) or only estimated them by indirect calibration (Zill and Moran 1981b). The following discussion, therefore, briefly reviews the results of previous studies in the context of the present findings.

Directional sensitivity of the tibial campaniform sensilla

The proximal and distal tibial campaniform sensilla responded with consistent directionality to all levels and rates of force applied to the tibia. These results fully support Chapman's conclusions that the receptors respond maximally to strains which act to compress the cuticular cap perpendicular to its long axis (Spinola and Chapman 1975). While these experiments only studied the encoding of forces imposed in the plane of joint movement, a previous study has shown that forces applied in this plane optimally excite the receptors (Zill and Moran 1981b). The actual distributions of

stresses that occur at the femoro-tibial joint during standing or walking in freely moving animals are unknown. The vectoral component of load, that is resisted by the actions of the tibial extensor and flexor muscles, occurs in the plane of joint movement. These results are therefore important in understanding the types of information that are provided by proprioceptors about the forces that the animal actively controls.

We have also found that the tibial sensilla show phasic discharges to decreasing forces (Ridgel et al. 1999). These types of responses occurred in many tests in the present series of experiments (Figs. 4, 6, 8) using ramp and hold stimuli, and they were ubiquitous in parallel studies we have performed using white noise as the driving waveform for forces applied to the leg (DiCaprio et al. 1998). Discharges during releases from applied forces are evident in earlier studies of campaniform sensilla of cockroaches (Spinola and Chapman 1975, Zill and Moran 1981b) and other insects (Delcomyn 1991; Newland and Emptage 1996). They also regularly occur in crustacean mechanoreceptors (cuticular stress detectors, Marchand et al. 1995). The mechanisms underlying responses to decrements in force are presently unknown.

In sum, these data support the hypothesis that *individual* tibial campaniform sensilla show a *single directional sensitivity* and provide precise information about force vectors and their resultant strains within the leg. In the tibia, the sensitivity to changing forces occurs in a preferred direction, in that sensilla encode both increasing strains in that direction and relaxation from forces applied in the opposite direction. As discharges to force decrements are only phasic, these findings also support the idea that the tibial campaniform sensilla are important detectors of changes in forces that act upon the leg.

Encoding of static load

We demonstrated that the discharges of the sensilla in the intervals early and late in the hold phase effectively encoded the level of force when averaged over time (Fig. 6). Previous reports of the tonic sensitivities of the cockroach tibial sensilla were based upon estimates of the magnitudes of forces applied to the tibia. Furthermore, Zill and Moran (1981b) compared the effects of force application in different directions during the first 100 msec after the stimulus onset. The present study has shown that this interval reflects the rate, and only secondarily the amplitude, of the applied stimulus. Thus, the plots of Zill and Moran (1981b) represent over-estimations of sensitivities of the tibial campaniform sensilla to applied loads.

Both the proximal and distal sensilla exhibit hysteresis in tonic discharges when forces are decreased from maximal levels. Hysteresis has been previously demonstrated in the trochanteral campaniform sensilla of the stick insect (Hofmann and Bässler 1986) and has been noted in a number of other sensory systems (vertebrates: Malbert and Leitner 1993; Segundo et al. 1995; invertebrates, Zill 1985a,b). The potential functions of hysteresis in sensory discharges are often unclear (Hatsopoulos et al. 1995) but Zill and Jepson-Innes (1988) suggested that it can be adaptive and reduce residual tensions in leg muscles.

We found that the extent of adaptation was considerable in the tibial campaniform sensilla of both orientations. Adaptation shown during single tests could be fitted to simple exponential functions (Chapman and Smith 1963; Bohnenberger 1981) but these functions had low correlation coefficients (r values) due to the considerable variation in the firing frequencies (Fig. 6). Recent studies (French and Torkelli 1994)

suggest that sensory adaptation in arthropod mechanoreceptors represents a sequence of processes with different time courses and that sensory responses are better described by a corresponding set of exponents rather than a single constant.

Thus, our experiments suggest that the tibial campaniform sensilla can effectively encode the static level of force, but the information provided by these receptors may be limited by extensive adaptation and hysteresis. It is important to note that all studies on the tibial group have only recorded the activities of the largest sensilla (Ridgel et al. 1999). Recordings of the discharges of the trochanteral campaniform sensilla have demonstrated that receptors of different spike amplitude show differential sensitivities to forces (Hofmann and Bässler 1986, 1982; Delcomyn 1991) and potentially range fractionation (Zill et al. 1999). Thus, it is possible that the tonic activities of smaller campaniform sensilla provide the system with information about static load that is less affected by hysteresis or temporal adaptation.

Encoding of rate of applied forces

All campaniform sensilla accurately encode the rate of force application to the leg (dF/dt) (Chapman et al. 1979; Hofmann and Bässler 1982, 1986). The afferent firing frequencies during ramp application of changing levels of force were, for the most part, much higher than those signaling tonic loads (compare Figs. 6, 8). The information indicating the rate of change of force may, therefore, be distinguishable by the system from discharges related to static levels of load. These findings support and confirm the results of Chapman and colleagues (1979) and Schnorbus (1971) which demonstrated that tibial sensilla could encode forces applied as sinusoidal stimuli up to 80-100 Hz. At those higher rates, responses showed saturation and receptors only fired single action

potentials per cycle of stimulation.

Responses to rates of change of force applied to the leg could be fitted with high correlation to simple power functions (Chapman et al. 1979; Thorson and Biederman-Thorson 1974), in contrast to our findings on receptor adaptation. Chapman, Mosinger and Duckrow (1979) also fitted the responses to cap indentation to power law functions (k coefficients, median = 0.38, range = 0.14-0.69). Furthermore, we examined the effects of varying the amplitude of the stimulus in sequences of tests of rate sensitivities. The power coefficients were larger for low amplitude forces but reached a constant value above a minimum level of amplitude. These findings imply that the receptor discharges unequivocally signal the rate of change of force when the magnitude of the forces are above a minimum threshold.

Campaniform sensilla of the legs are, therefore, particularly tuned to monitor changing levels of force in ranges that occur during locomotion and postural perturbations. In parallel studies, we have found that the sensitivity of the afferent firing frequency to the rate of force application is also present when mechanical stimuli are applied to the leg using waveforms generated as white noise (DiCaprio et al. 1998). However, no studies to date have systematically examined whether information is provided by the receptors through temporal coding in the patterns or trains of action potentials that are generated in responses to changing forces. Studies by Chapman using cap stimulation have suggested that the sensilla may produce the largest number of spikes at particular frequencies of sinusoidal stimulation, and this is clearly an area for future research (Chapman et al. 1979).

Effects of Pre-loading

A major finding of the present study was that the tonic responses of all tibial campaniform sensilla were altered by application of prior static loads while encoding of the rate of change of forces was unaffected. These findings are important because the leg must regularly be used to support the animal's weight in posture and locomotion. Variations in forces that accompany perturbations would therefore occur as changes about this static level. To our knowledge, no other studies on campaniform sensilla have examined responses after application of tonic loads to the leg. Chapman, Mosinger and Duckrow (1979) recorded the responses of the tibial receptors to sinusoidally varying forces applied to the cuticular cap. Of necessity, these forces were applied at an initial static offset level of force. All parameters measured (indentation, force sensitivity and cap compliance) were similar when forces were applied at static offset levels of 10-15 microNewtons. Measurements below this offset were difficult to control for technical reasons. (However, one sensillum showed very non-linear responses in this region). These results are similar to those obtained in the present study, if the tonic force applied to a leg produces a continuous indentation of the cuticular cap. Juusola and French (1995) recently studied the dynamic responses of sensilla of the spider VS-3 lyriform organ (Seyfarth and French 1994) by application of white noise stimuli to the cuticle overlying the slit. They found that response sensitivities to changing forces were maintained at all initial offset levels of force. Those results clearly parallel the present findings (Fig. 9), but it is not yet known whether the rate sensitivities of slit sensilla are comparably preserved when forces are applied to the legs of spiders.

In contrast to sensitivity to rate of change of force, static sensitivities of the receptors were altered by imposition of prior loads. The effects of pre-loading on tonic afferent activities were to effectively shift the range of responsiveness of the receptors so that their firing frequencies were decreased relative to the unloaded condition. The sensitivities to the levels of force were, apparently, reset after application of a continuous static load. The potential causes of these effects are unknown, but could lie in properties of the mechanisms of neuronal adaptation. For example, French (1989) studied the effects of holding currents applied extracellularly to the sensory neuron of the cockroach femoral spine. These currents were applied near the axo-somatic junctional region, close to the presumed action potential generator. He demonstrated that the effect of prior injection of depolarizing currents was to raise the threshold level of depolarization that was needed for subsequent generation of spikes. This type of mechanism could account for the shift in the range of sensitivities of the cockroach tibial campaniform sensilla if forces applied to the leg produced continuous depolarizations through the generator potential mechanism (Mann and Chapman 1975). Furthermore, Juusola and French (1995) studied (via intracellular recording) responses of single neurons of spider slit sensilla to mechanical stimuli applied as step displacements to the cuticle. Increases in force were applied to the slit in the presence of tonically maintained forces. The effect of static strains was to shift the neuron's sensitivities (measured as peak depolarizing currents) which 'reset the neuron's operational range' (Juusola and French 1995). Those results are qualitatively similar to the changes in sensitivity to force level we have demonstrated for the tibial campaniform sensilla and imply that one source of the shifts in sensitivity could be the adaptation properties of the

action potential generating mechanism.

Differences in responses of proximal and distal sensilla

The proximal and distal tibial campaniform sensilla showed consistent differences in their response properties in all tests of static and dynamic sensitivities. The proximal sensilla fired at higher frequencies than the distal receptors at equivalent levels and rates of applied force. The distal sensilla also had higher thresholds and adapted more rapidly to maintained forces. The sensitivities (and exponential coefficients) to force rate were higher for the distal than for the proximal sensilla (Table 1). In contrast, previous studies using cap stimulation found no differences in thresholds or sensitivities between the subgroups of tibial sensilla, either in their spiking discharges (Chapman and Duckrow 1975) or in recordings of generator potentials (Mann and Chapman 1975). Thus, the differential sensitivities we observed apparently resulted from application of forces to the leg, rather than the cuticular caps.

One possible source for these differences could be in the distributions of strains in the cuticle of the tibia. The distal sensilla are activated by compressive strains oriented perpendicular to the tibial long axis, which are probably lower than the axial compressions which excite the proximal receptors (Hibbeler 1993; Cocatre-Zilgien and Delcomyn 1999). While the tibia has been modeled as a cylinder (Zill and Moran 1981b), it possesses a number of large cuticular spines that are distinctly asymmetrical in distribution (Chapman 1965). Asymmetry in the degree of sclerotization and resultant mechanical properties has been demonstrated in the proximal tibia of the locust hindleg by Heitler (1974) and is considered to play a role in the mechanism of jumping (Heitler and Burrows 1977; Burrows 1996). While a comparable difference in

sclerotization is not apparent in the cockroach tibia, our preliminary morphological investigations have shown that the cuticle of the tibia in the region of the sensilla is thinner than surrounding areas (see Flannigan 1998 for comparable results in the cockroach trochanter). These types of changes in wall thickness could lead to substantially higher compressive stresses when forces are applied which bend the distal tibia and should be incorporated in future models of the strains that excite the tibial receptors.

Comparison with campaniform sensilla of insect wings

Campaniform sensilla of the wings of insects have also been shown to encode dynamic changes in forces but they differ significantly from sensilla of the legs in their directional sensitivity and the rates of forces they can signal. Individual campaniform sensilla of the wings of flies can exhibit *bi-directional* responses and discharges to increasing forces during both dorsal and ventral bending of the wing (Dickinson 1992). Their morphology also differs from campaniform sensilla of the legs, in that the cuticular cap is not ovoid but only slightly elliptical (Dickinson 1992). The surrounding cuticle lacks the thickenings (buttresses) that may provide mechanical coupling for compressive strains and the dendrite is circular (it is flattened in leg receptors, Moran and Rowley 1975). Wing campaniform sensilla respond to dynamic forces over an even broader range of rates than receptors of the legs. Insect flight occurs at much higher frequencies of movement than the legs are cycled during walking (maximum 25 cycles/second) (Dickinson 1990a, Full and Tu 1991). At these high rates, individual wing sensilla probably fire only single action potentials during one cycle of wing movement and can only function as 'event' detectors (Dickinson 1990b). Thus,

campaniform sensilla of both the wings and legs are excellent detectors of force dynamics and they are specifically tuned to the frequencies of movement of their respective appendages. Furthermore, the directional sensitivity of receptors of the legs may reflect the need to resolve the direction of force vectors in a multi-legged animal in posture and locomotion.

Comparison with other force receptors

In a number of invertebrates and vertebrates, receptors that monitor forces in the legs have also been shown to be sensitive to the rate of change of force. Slit sensilla of legs of spiders (Seyfarth et al. 1985; Seyfarth and French 1994), for example, encode force dynamics and also show considerable adaptation to stimuli applied as step increases (Barth and Bohnenberger 1978; Barth 1981; Bohnenberger 1981; Blickhan and Barth 1985; Seyfarth and French 1994). Responses of individual slit sensilla to step and sinusoidal functions can be described by simple power law exponents, that are similar in value to those calculated in the present study (Bohnenberger 1981). In crustacea, cuticular stress detectors (CSD) and funnel canal organs encode strains in the exoskeleton that result from both external loads and muscle contractions (Marchand et al. 1995; Libersat et al. 1987a,b; Zill et al. 1985) and individual units encode specific ranges of dF/dt (Marchand et al. 1995).

Internal receptors that monitor forces as muscle tensions have also been shown to be rate sensitive. Tryba and Hartman (1997) recently demonstrated that apodeme receptors of the crab opener muscle are specifically sensitive to the rate of change of force development and firing to active muscle contractions is maximal at peak force velocity, not at the highest level of force. Davies et al. (1995) showed that the initial

sensory discharge of Golgi tendon organs was strongly dependent upon the rate of development of force. Plots of the mean firing rates of tendon organs during the rising phase of muscle tensions (prior to the attainment of plateaus) showed an exponential relationship between sensory activities and dF/dt . Other studies have also demonstrated that the responses to changing forces are maintained over a wide range of static tensions (Jami et al. 1985). Davies et al. (1995) conclude that 'the dynamic component of the tendon organ's responses dominates the level of afferent discharge, irrespective of the absolute tension'. These findings suggest that the sensitivities to the rate of change of force may also be preserved in the presence of static load in other systems, as well as in cockroach tibial campaniform sensilla.

Potential benefits of information about force dynamics in control of posture and locomotion

Recent studies of the kinetics of cockroach locomotion suggest that the sensitivities of the tibial campaniform sensilla to force dynamics should strongly contribute to the signals they provide during walking (Full and Tu 1990; Full et al. 1991). The ground reaction forces exerted by limbs during locomotion in both cockroaches (Full and Tu 1991) and vertebrates (Dietz 1998) are not constant but show ongoing changes as load is applied and propulsion is generated during stance. Zill and Moran (1981b) recorded activities of the tibial campaniform sensilla during walking. They found that the proximal receptors fired early in the stance phase following leg contact, in a burst that was initiated at a high level. During this time, ground reaction forces rise rapidly, as loading is increased and the leg begins to exert force in support. The dynamic sensitivity of the proximal sensilla could serve to signal the rate of leg loading early in stance. These

inputs could also appropriately adjust the activities of leg extensor muscles to the rate of force development through their known reflex effects (Zill et al. 1981).

Force plate measurements have also shown that ground reaction forces rapidly decline in the latter half of the stance phase (Full and Tu 1991). The present study has confirmed that campaniform sensilla can show vigorous discharges to declining levels of force. These bursts can also encode the rate of force decrease (Ridgel et al. 1999). The distal tibial campaniform sensilla fire in a short burst at the end of stance (Zill and Moran 1981b). This activity could readily result from rapid decreases in strains as the leg is unloaded. It may also assist in determining the phase of the onset of the swing phase through reflex activation of flexor motoneurons (Zill et al. 1981). We have previously tested these hypotheses by examining responses to bending forces using waveforms that approximated the durations and magnitudes of ground reaction forces that occur during cockroach walking (Full and Tu 1991). Stimuli applied in the direction of extension as simple, rapid triangle functions elicited reciprocal discharges in the subgroups of tibial sensilla similar to that seen in walking (Ridgel et al. 1999, Fig. 2G). Future experiments are planned to further extend these tests by utilizing force patterns directly derived from measurements of ground reaction forces. The present findings suggest that the tibial campaniform sensilla could function to adjust the magnitudes and phase of motoneuron activation by monitoring the cyclical development and decline of forces in the leg.

Lastly, the dynamic sensitivities of the tibial campaniform sensilla could aid in the generation of reactions to perturbations. During platform perturbations, changes in forces upon limbs precede changes in joint angles or body position (Macpherson

1988a,b) and the magnitude of compensatory reactions also depend upon the rate of force application (Diener et al. 1988, Burleigh and Horak 1996). In addition, large forces or those applied at rapid rates can elicit compensatory steps, in which a leg is lifted and moved to a new location (Maki et al. 1993; Zill 1993). These stepping reactions reposition the legs to provide support for the center of gravity or center of pressure (Burleigh and Horak 1996; Mcilroy and Maki 1994; Burleigh et al. 1994; Mcilroy and Maki 1993). In these cases, receptors monitoring the rate of change of forces may function as discrete signals indicating that swaying reactions would be insufficient and that rapid limb movements were necessary. In conclusion, we suggest that these potential functions of receptors that encode the rate of change of force may be advantageous in both biological systems and robotic applications.

CHAPTER 3

**RESPONSE PROPERTIES OF TIBIAL CAMPANIFORM SENSILLA STUDIED BY
SUBSTRATE DISPLACEMENT IN FREELY MOVING COCKROACHES**

SUMMARY

We characterized the responses of the cockroach tibial campaniform sensilla, receptors that encode mechanical strains in the exoskeleton of the leg, by recording sensory activities during postural perturbations in freely standing animals. The entire substrate upon which the animal stood was displaced horizontally using ramp and hold stimuli at varied rates under computer control. The tibial sensilla showed discrete, short latency responses that were initiated in the first 30 ms of platform movement. Responses of individual receptors depended upon the direction of the displacement and the orientation of the receptor's cuticular cap. Proximal receptors, whose cuticular caps are normal to the long axis of the tibia, responded to displacements directed from the contralateral side of the body and from the head toward the abdomen. The distal sensilla, oriented parallel to the tibia, discharged at longer latency to displacements in the opposite directions. Plots of afferent firing frequencies versus displacement direction (relative to the tibia) showed that proximal and distal sensilla are activated in non-overlapping ranges of movement direction. Furthermore, afferent responses also reflected the rate of displacement and both maximum and mean firing frequencies increased as the platform was displaced more rapidly. Despite the complexity of distal leg structures that transmit forces from the substrate, these results are consistent with a model in which displacements produce forces that result in bending of the tibia. The response properties of the receptors could contribute to the detection of the direction and rate of forces that occur during leg slipping or in walking on unstable terrains.

INTRODUCTION

The forces that occur in the legs of an animal during standing and walking can be derived from a number of diverse sources, including body loading, inertia and the contractions of limb muscles. It has, therefore, proven difficult to accurately evaluate the specific parameters encoded by sense organs that monitor these forces. For example, in vertebrates, forces on the limbs are thought to be encoded via activities of Golgi tendon organs (Prochazka 1996). Studies in restrained cats have shown that the firing frequencies of these receptors reflect the magnitude of loads applied to the muscle tendon (Houk and Henneman 1967; Fukami and Wilkinson 1977; Jami 1992). However, tendon organ afferents also encode the force produced by muscle contractions and they are highly sensitive to the rate of tension development (Prochazka and Wand 1980; Davies et al. 1995). Recordings obtained in walking have shown that tendon organs are active maximally during the stance phase when loads are applied to the legs (Loeb 1981, Appenteng and Prochazka 1984, Prochazka and Gorassini 1998a,b). However, it is not clear to what extent these discharges reflect the forces resulting from body loading or the magnitude of contractions of leg muscles that contribute to support and propulsion. Furthermore, few studies have recorded the activity of tendon organ afferents in freely moving animals during perturbations of posture (Aniss et al. 1990). While existing data suggest that discharges of the tendon organs can reflect both active and passive forces at the junction between the muscle and the tendon, the specific way that Golgi tendon organs encode these forces in freely standing animals has not yet been determined.

In invertebrates, forces acting upon the leg are monitored by receptors located at the insertions of muscles or by sense organs associated with the exoskeleton. These types of sense organs also respond to passive loading and to forces generated by muscle tensions. In crustaceans, force-sensitive mechanoreceptors in the dactyl (foot) of the crab are active in the stance phase of walking and are silent during swing (Libersat et al. 1987a,b). The firing frequencies of these receptors during stance increase significantly when weights are added to the crab's body, demonstrating that the receptors encode forces that result from body load. However, force-sensitive mechanoreceptors also fire vigorously to contractions of leg muscles when leg movements are resisted (Libersat et al. 1987a). These forces could contribute to or bias the discharges to leg loading. Similar responses to forces on the legs during locomotion have been described in cuticular stress detectors in crayfish (Marchand et al. 1995, Klärner and Barth 1986, Klärner and Barnes 1986).

In insects, forces on the legs are detected as strains in the exoskeleton by campaniform sensilla (Pringle 1938b; Hofmann and Bässler 1982, 1986; Delcomyn 1991; Schmitz 1993; Zill and Seyfarth 1996). The strains are monitored via small cuticular caps that form the attachment points for the sensory dendrites (Moran et al. 1971). Previous studies have characterized the encoding of forces by campaniform sensilla of the tibia (Group 6 of Pringle 1938b) in restrained preparations and have demonstrated that the receptors show directional sensitivity to bending of the tibia (Zill and Moran 1981a, Ridgel et al. 1999, 2000). The phasic component of the discharges of the sensilla also strictly encodes the rate of applied force (dF/dt). However, those experiments (and previous works by Schnorbus 1971, Spinola and Chapman 1975)

were performed with the femoro-tibial joint immobilized and forces were applied directly to the tibia. It is not known if forces applied through the tarsus would elicit similar responses from the receptors. While the load was applied to the leg at levels within the range of those measured as ground reaction forces during running (Full and Tu 1990, 1991), the exact magnitude and orientation of forces exerted by the hind leg in walking are not available. Therefore, it is not clear if these applied forces adequately replicated those occurring during posture and walking. Lastly, the tibial muscles were denervated in studies in restrained preparations. These muscles are known to exert strong direct influences upon the activities of the tibial campaniform sensilla (Zill and Moran 1981a,b;; Zill et al. 1981), as has also been shown in the trochanteral campaniform sensilla in stick insects (Delcomyn 1991) and in model studies in cockroaches (Ramasubramanian et al. 1999; Flannigan 1998; Flannigan et al. 1998). These diverse and potentially complex sources of forces could bias or dampen the responses of the tibial sensilla to variations in load when animals are walking or standing.

Zill and Moran (1981b) first developed techniques for recording the activities of the tibial campaniform sensilla in freely moving animals. In these experiments, the receptors were shown to be active during the stance phase of walking. The discharges were thought to reflect cuticular strains resulting from two sources, body loading and the contractions of leg muscles. These hypotheses have not been tested in freely standing animals using techniques to alter force on the legs, such as substrate perturbations.

In studies described in this report, we utilized ramp and hold displacements of the substrate to evaluate the response properties of the tibial campaniform sensilla in unrestrained animals. Cockroaches stood freely within the test arena and could

spontaneously assume a variety of postures or orientations relative to the direction of platform movement. In order to examine the dynamic sensitivities of the receptors, the rate of rise of the ramp phase was increased within a series. Our studies have shown that the tibial receptors show discrete, short latency responses immediately following the onset of platform movement. The activity of individual sensilla depended upon the orientation of the cuticular cap and varied according to the rate of displacement. These experiments suggest that the tibial receptors can provide the nervous system with information about the direction and rate of perturbations which could be utilized to detect leg slipping or in traversing unstable terrain.

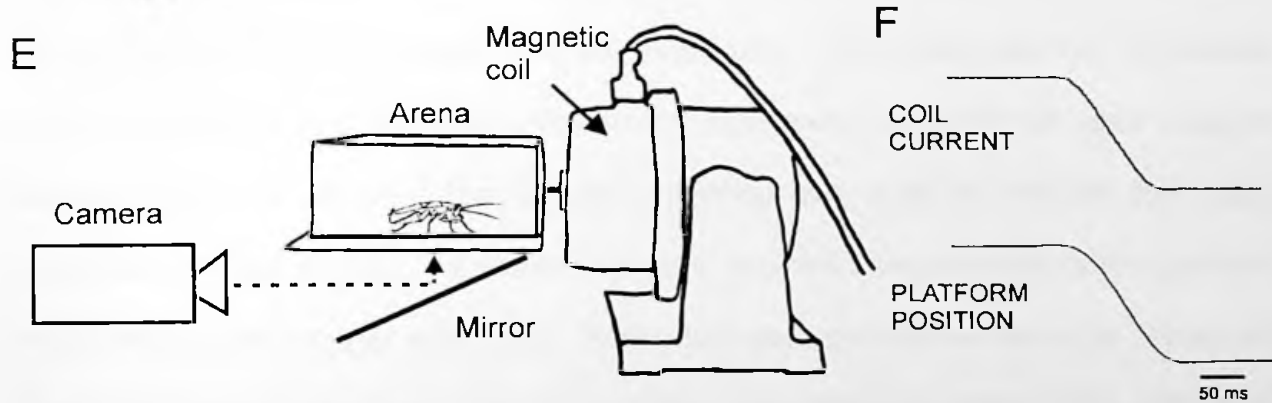
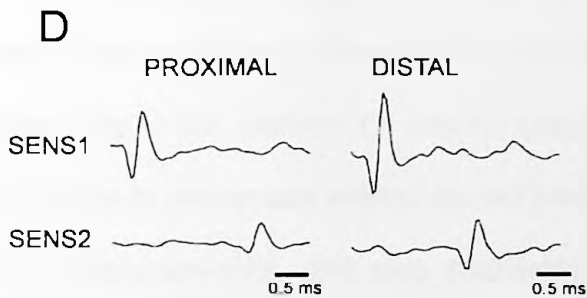
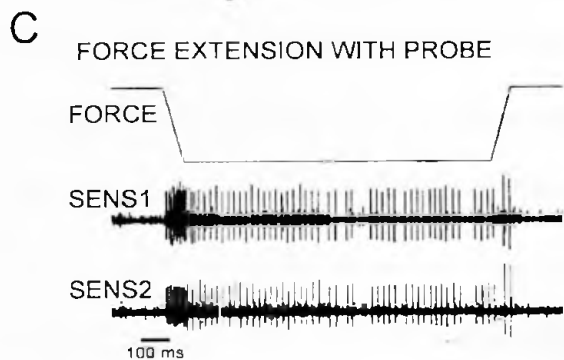
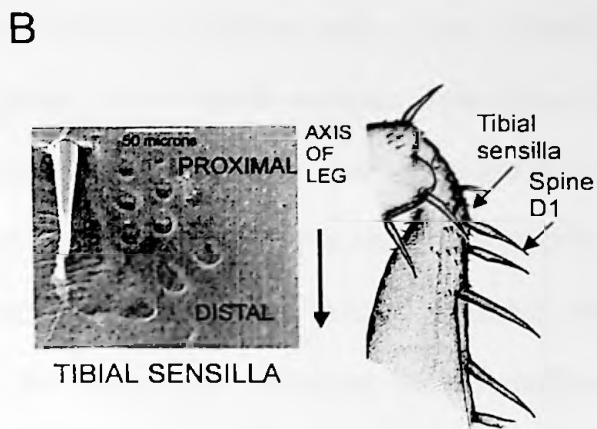
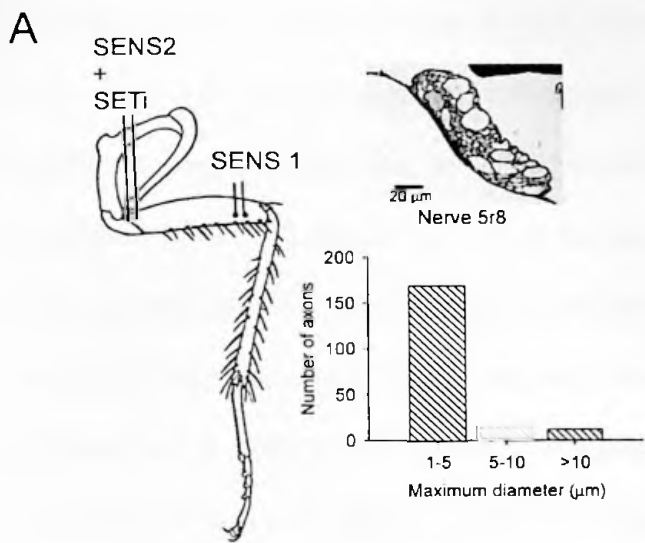
MATERIALS AND METHODS

Freely moving animals

Adult male *Periplaneta americana* (n= 29) were first anaesthetized with carbon dioxide and secured, ventral side up, to a Sylgard resin-coated block using staples made from insect pins. To record activities of the tibial campaniform sensilla, a pair of 50 μm silver wires was inserted near nerve 5r8 (Nijenhuis and Dresden 1956) in the femur of the left metathoracic leg (SENS1, Fig. 11A). This nerve is purely sensory and contains axons derived from the largest tibial campaniform sensilla and from tactile spines on the femur and proximal tibia (Figs. 11A,B) (Zill et al. 1980). A light micrograph of a histological section of nerve 5r8 (following the methods of Zill et al. 1980) shows that it contains a limited number of larger diameter fibers ($>10 \mu\text{m}$, Fig. 11A). Electrodes were inserted into the femur while using a probe (driven by a piezoelectric crystal) to bend the distal tibia. Bending of the leg produced consistent discharges from the receptors. The wires were positioned to maximize the size of the

Figure 11: Experimental Setup. **A** The activity of the tibial campaniform sensilla was recorded in the femur of the left metathoracic leg with two pairs of wire electrodes. One pair of electrodes was positioned near nerve 5r8 (SENS1) and the other was implanted near the trochanter-femur joint (SENS2). The second set of wires also recorded myographic activity from the tibial extensor muscle (SETi). The inset shows a light micrograph of a cross section through nerve 5r8, which contains only sensory axons. The graph is a histogram of the distribution of axon diameters in the nerve. Nerve 5r8 has a small number of axons greater than 10 μm which are derived from the tibial sensilla and the tibial spines. **B** The campaniform sensilla are located in the proximal end of the tibia and consist of two subgroups, proximal and distal receptors. A scanning electron micrograph shows that the long axes of the cuticular caps of the distal sensilla are oriented parallel to the axis of the leg while the proximal receptors are oriented perpendicular to the leg. The tibial spine, D1 (as termed by Chapman and Pankhurst 1967), also has its axon in nerve 5r8 and was stimulated during the experiment as a control (see Fig. 12B). **C** Force was applied to the tibia (force extension) with a probe while positioning the electrodes to obtain a maximal signal from the receptors. Typically, a small unit (proximal sensillum) was recorded upon force extension while a large unit (distal) was activated upon release of force. **D** The action potentials were first recorded on the wires near the femoro-tibial joint (SENS1) and then, after a short delay, on the electrodes near the trochanter-femur joint (SENS2). The direction of action potential propagation confirmed that recordings taken during perturbations were sensory. The distance between the two pairs of wires was measured and used to calculate the conduction velocities of the sensilla (distal, 5.9 ± 0.9 m/s; proximal, 4.8 ± 0.8 m/s). The

units recorded during platform displacements had the same conduction velocities. **E** After the recording electrodes were secured, the animal was released into an arena that was attached to a magnetic coil. High-speed video images (250/500 frames/sec) recorded the animal position and orientation from below (via the mirror). **F** Displacements of the arena were generated by applying voltages to the magnetic coil using a computer. Movement of the platform, as monitored from digitized video images (PLATFORM POSITION), was closely correlated the current to the coil (COIL CURRENT).



extracellularly recorded action potentials (100-150 microvolts). Typically (but not invariably), the activities of one or two proximal and one distal receptor were recorded (Fig. 11C) and the extracellularly recorded spikes of the distal sensilla were larger in amplitude than the proximal sensilla. A second pair of wires was inserted in the femur adjacent to the trochanter-femur joint to record from the axons of the receptors near the point that nerve 5r8 joins the main trunk of nerve 5 (SENS2, Fig. 11A). The use of two recording sites allowed us to confirm that the responses obtained during platform displacements were conclusively sensory since spikes were first recorded on the distal electrodes in the mid-femur and then on the more proximal wires near the trochanter-femur joint. The sensory action potentials were conducted between the pairs of recording electrodes with a measurable delay (Fig. 11D, mean= 1.1 ms for proximal sensilla, 0.8 ms for distal sensilla) that was identical in responses elicited by leg bending and in activities that occurred during platform displacements. We also calculated the conduction velocities of the sensilla (proximal = 4.8 ± 0.8 m/s, distal = 5.9 ± 0.9 m/s) by measuring the distance between the electrode sets. The wires near the trochanter-femur joint also recorded the activities of the slow motoneuron of the tibial extensor muscle (Fig. 11A, SETi). The muscle recording was used to monitor the active movements of the animal. All electrodes were secured using cyanoacrylate glue and tied to the leg with copper wire (#40). Small dots were painted on the coxa, femur and tibia of both metathoracic legs and the abdomen with white nail polish. The wings were trimmed in order to minimize contact with the wires.

In order to test the responses of the receptors in freely standing animals during substrate perturbations, we placed individuals in an arena that had a mesh or Plexiglas

floor (Fig. 11E). The area of the arena was large enough (20 X 9 mm) for the animal to walk freely. The chamber was mounted to the moveable arm of a large magnetic coil (Ling Dynamic Systems, Model V408, Fig. 11E) and positioned in a horizontal plane (checked with a level). Ramp and hold waveforms were applied to the coil via computer. These waveforms were generated using Datapac II software (Run Technologies) and filtered at 40 Hz (Brownlee Precision, Model 440) to decrease the rate of rise and termination of the ramp. This level of filtering was established by testing the coil and arena until overshoot and endogenous oscillation was minimized. The waveforms were amplified (APS Dynamics, Model 124) and used to displace the chamber. The arena first moved away from the coil, and held in that position for 1 second. It was then displaced in the opposite direction at the same rate. Six perturbations were applied that increased in rates of rise and decline of the ramp throughout the series. The amplitude of displacement was held constant within a set of perturbations but could be varied experimentally (1-8mm) in different tests. Displacement of the platform was monitored with a Sandeman transducer or by a linear potentiometer linked to the chamber. We also recorded the current of the magnetic coil (Fig. 11F, Coil current) and measured the distance of chamber movement via high-speed video images that were taken during the experiments (Fig. 11F, Platform position).

Our protocol was to first record sensory activities during bouts of walking (Fig. 12A) and then to initiate series of tests of platform perturbations (Figs. 13, 14 and 16). As a control, we also mechanically stimulated (using a hand-held probe) the first tactile spine on the tibia (spine D1, Chapman and Pankhurst 1967) while the animal was standing in

Figure 12: Experimental protocol and controls. Sensory activity was first recorded during walking and then in tests of platform perturbation. **A** During walking, the proximal sensilla fired early in the stance phase (small amplitude unit, SENS1) in bursts that were initiated prior to the onset of slow extensor activity (SETi, large unit on PROX FEMUR). The activities of the proximal receptors were largely reciprocal with firing of the SETi. The distal sensilla fired at the end of the SETi burst (large unit on SENS1). **B** Tibial spine D1 was activated when stimulated with a hand-held probe. **C** After the perturbation tests, the animal was restrained and the tibial sensilla were ablated with a fine tungsten wire, producing an injury discharge of large and small units. The amplitude of these discharges was similar to those recorded during the perturbation experiments. **D** The absence of sensilla activity upon leg bending confirmed that the receptor ablation was successful. **E** The animal was once again released into the arena and walking was recorded. Activity of the tibial sensilla was not present on the sensory recording; however, the slow extensor tibial muscle was still active during walking. **F** Stimulation of spine D1 confirmed that the nerve recording was still intact and that the ablation was localized to the campaniform sensilla.

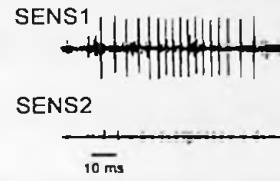
A

ACTIVITY DURING WALKING



B

ACTIVITY OF SPINE D1



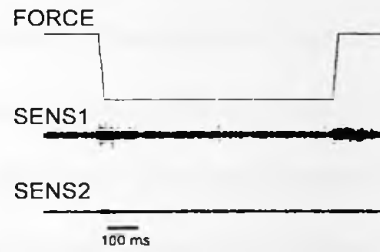
C

ABLATION OF TIBIAL CS



D

POST-ABLATION FORCE EXTENSION



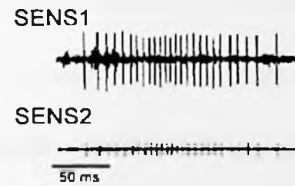
E

POST-ABLATION WALKING



F

POST-ABLATION ACTIVITY OF SPINE D1



the arena. This elicited a high frequency burst of unitary amplitude in the sensory recordings (Fig. 12B). Animals were then restrained and the responses to bending of the tibia were retested (data not shown). The cuticular caps of individual sensilla were stimulated using a fine wire probe to identify the receptors. The caps were subsequently ablated using a fine tungsten wire or a minuten pin. This ablation resulted in an intense discharge (Fig. 12C) of units that had similar amplitudes to those recorded during leg bending. The small hole in the cuticle that resulted from the ablation could be visualized under a dissecting scope to confirm that the specific region of the caps had been penetrated. Following receptor ablation, forces were applied to the tibia in the direction of forced extension (using the force probe) to verify that the sensory discharge had been eliminated (Fig. 12D). Animals were again released into the test arena. The sensory activities previously recorded during walking and in postural perturbations were absent (Fig. 12E). However, stimulation of the tactile spine D1 with the probe showed that its activity could still be recorded following sensilla ablation (Fig. 12F). This control demonstrated that the ablation was highly localized and did not damage the sensory neuron of the spine. It also confirmed that the spine itself did not contribute to the discharges elicited during postural tests.

In a few preparations (n=4 of 29), we ablated the tibial spines (except for the spine D1) and the spines on the bottom of the femur before releasing the animal into the arena (data not shown). This control insured that the tactile spines were not contributing to the sensory response recorded during the experiments. The afferent activity recorded in these tests was similar to that seen in intact animals.

High-speed video images (Redlake video camera, 250 or 500 frames/sec) of the animal were taken with a single camera that contained a view from below the arena, via a mirror mounted at 45 degrees under the test chamber (Fig. 11E). These videos were downloaded at 30 frames/s and stored on videotape. Only tests in which the animal held its posture throughout the series and did not walk were analyzed. While we were unable to control for the amount of body weight that an animal applied to the leg, tests in which the leg was held elevated from the substrate were not analyzed. Two-dimensional segmental angles of the femur-tibia joint were calculated by digitizing the position of the leg dots from the video images using Motus 4.3 software (Peak Performance). In addition, the orientation of the tibia relative to the direction of displacement was calculated using captured video images and Image Pro 3.0 software (Media Cybernetics). All stimuli and sensory recordings were amplified and stored on a TEAC recorder on digital audio tape (DAT). Experiments were transcribed from tape to computer using a CED 1401 analog to digital converter and afferent frequencies were calculated in Spike2 3.0 software (Cambridge Electronic Design). Joint angle measurements were synchronized with the physiology data using a synch pulse that was recorded on both the video and the physiology recordings. Statistics were calculated using StatView 5.0 (SAS Institute).

Restrained preparations

Animals (n= 5) were restrained, ventral side up, on a Sylgard resin-coated block (similar to that described previously) and two pairs of wires were inserted near nerve 5r8 in the middle of the femur. The nerves innervating the left metathoracic leg (Nijenhuis and Dresden 1956) were cut under the cuticle near the metathoracic ganglia

to eliminate motor activity. The tarsus was then severed and the spines on the end of the tibia were cut. A small drop of sealing wax was placed on the end of the tibia in order to minimize slipping of the bending device. Forces were imposed upon the distal end of the tibia using a force probe and were monitored with a custom-built strain gauge amplifier. In some of the tests, the end of the tibia was waxed to the force probe to provide a stiff linkage between the leg and the device. At the end of an experiment, individual campaniform sensilla were identified as previously described (Ridgel et al. 1999, 2000).

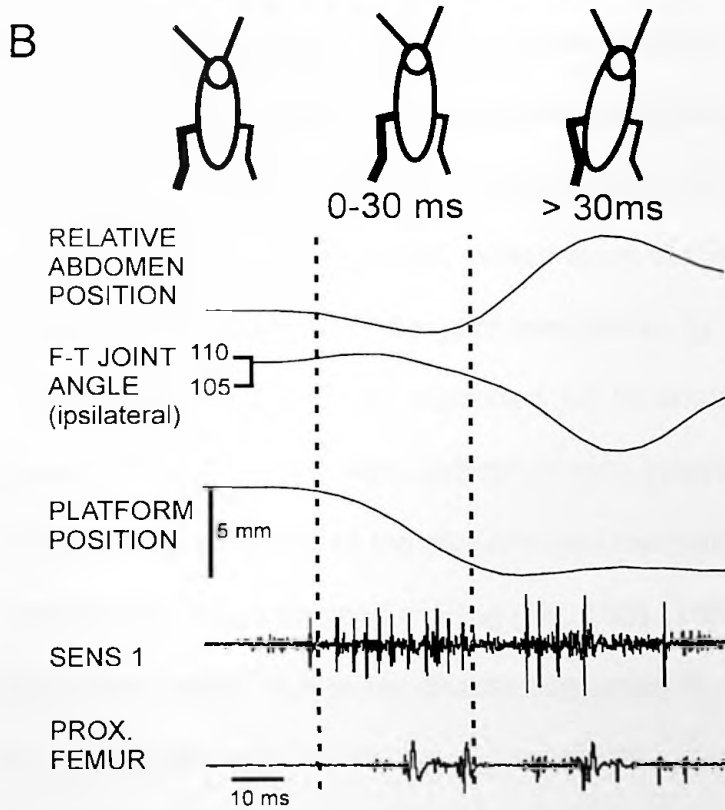
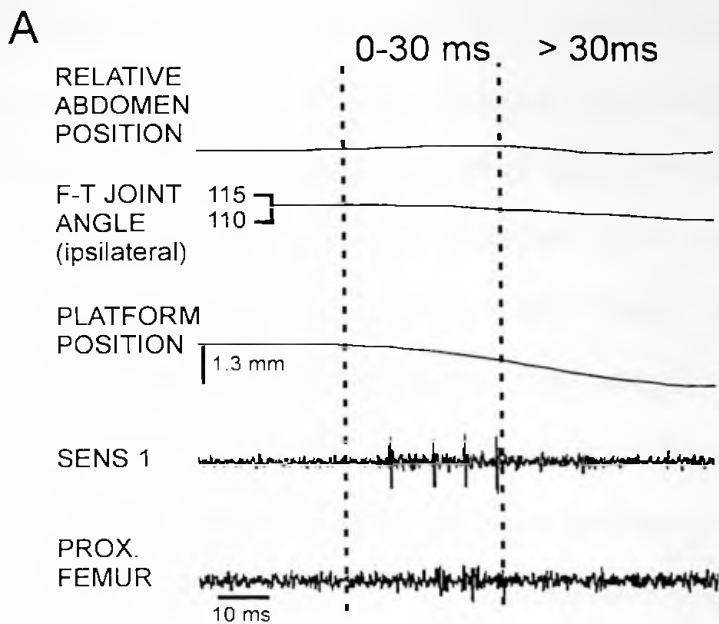
RESULTS

General description of responses to platform perturbations

Cockroaches were readily able to maintain stable postures when the substrate upon which they were standing was displaced. Escape responses, characterized by rapid turning and running, were not elicited in any tests. Although the platform moved rapidly during ramp perturbations, the distance that it was displaced was relatively small (1-8 mm) and the tarsi were not observed to lose contact with substrate or to slide along the surface. Furthermore, the imposition of perturbations did not evoke walking movements, although individual legs could be lifted and repositioned following the start of a test sequence. Data were analyzed after these adjustments had occurred and stable postures were maintained.

Swaying responses, consisting of shifts in the position of the abdomen and thorax and changes in leg joint angles, were apparent during many platform displacements. In 26 ramp perturbations (N=5 animals), the dots on the abdomen, the legs and the arena were digitized. The position of the abdomen, the angle of the femoro-tibial joint of the

Figure 13: Kinematic data and sensory responses during platform perturbations. Animals were placed in an arena which was displaced a constant distance using a series of ramp and hold waveforms. Relative abdomen position (difference between position of the abdomen and the platform), F-T joint angle (ipsilateral leg) and platform position were calculated from markers on the body and legs. **A** During a low amplitude perturbation (1.3 mm), the proximal sensillum discharges during the first 30 ms of platform movement. Only slight changes in the abdomen position and femoro-tibial joint angle were seen. **B** The proximal sensillum discharges at a higher rate during the first 30 ms of a larger and more rapid perturbation. In this test, activation of the tibial extensor occurs toward the end of this period. Changes in joint angles and relative body position are also apparent. A small extension of the joint (2 degrees) occurs in the initial period of platform movement, which is followed by a larger joint flexion (9 degrees) later in the substrate translation. The joint flexion is concurrent with a shift in the abdomen position. The distal sensilla showed a discharge that occurs after the platform movement ceased. **Top of B-** Schematic diagrams of body and leg position, as traced from video images, during a 5 mm ramp perturbation.



hindleg and the movement of the platform were calculated from the digitized points. In low amplitude perturbations, very little change in abdomen position or in leg joint angle occurred (Fig. 13A, 1.3 mm). However, body sway could be detected in video images of larger amplitude displacements (Fig. 13B, 5 mm, top). During the early period of platform movement, there were small deviations in the abdomen position and femoro-tibial joint angle (mean change = 2.4°) (Fig. 13B). These changes were not apparent in all tests (N= 21 of 26 ramp perturbations), but typically occurred as joint extensions when displacements were directed perpendicular to the body axis from the contralateral side and joint flexions when translations were ipsilateral. However, larger joint angle changes (mean = 6.5° , N= 26 ramp perturbations) were evident later in the ramp displacement (>30 ms) and following the cessation of the perturbation that were of opposite sign to the earlier deviations. In perturbations directed perpendicular to the body axis, the femoro-tibial joint was flexed when the platform displacement was from the contralateral direction and extended when the substrate moved from the ipsilateral side. During displacements parallel to the body axis, the joint was flexed by displacements directed from the head toward the abdomen and extended by translations in the opposite direction. These changes in joint angle were coincident with swaying of the abdomen. Initially, it lagged behind the movement of the platform and then continued in the direction of displacement after the substrate stopped moving (Fig. 13B). The abdomen represents about 35% of the total body weight and is not directly supported by the legs. The undamped movement of the abdomen could readily have caused changes in joint angles that occurred toward the end of ramp displacement if it acted as a pendulum.

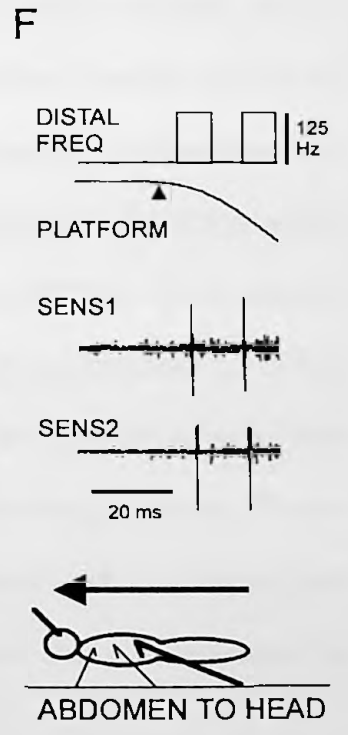
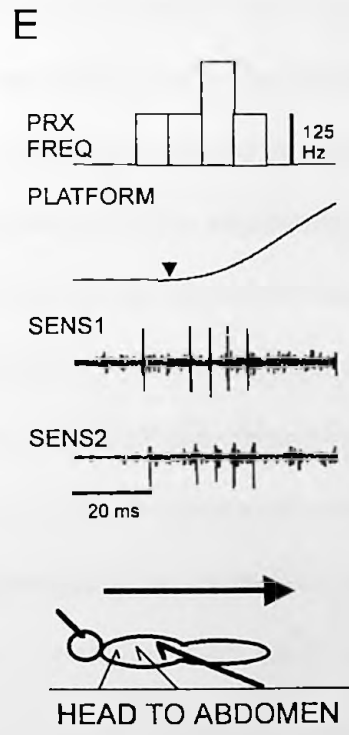
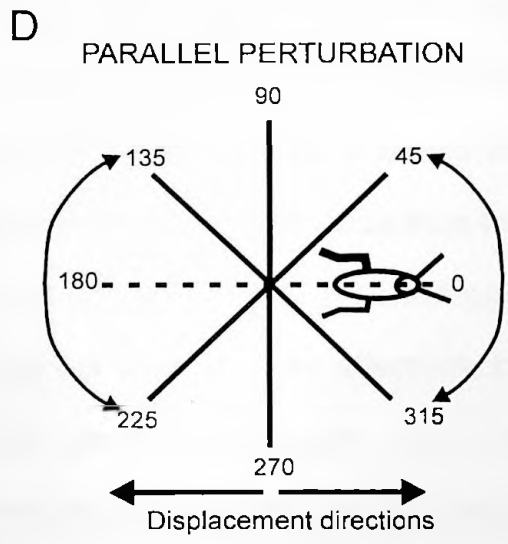
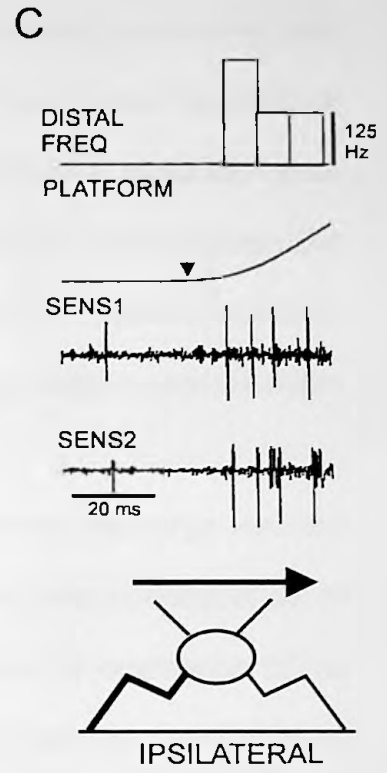
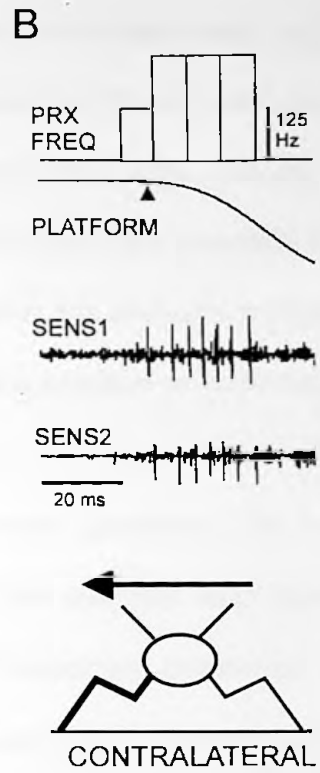
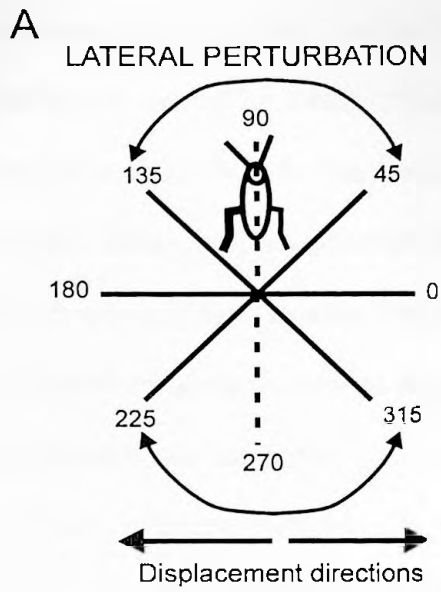
Sensilla responses to platform movement

The tibial campaniform sensilla fired bursts of activity following the onset of platform displacement. This study focuses upon the sensory discharges that were initiated within the first 30 ms, as body sway and recorded muscle activities were minimal during this time period. The discharges of individual sensilla depended on the orientation of the receptor's cuticular cap and the direction of platform movement. As a frame of reference, we measured the direction of displacement relative to the long axis of the tibia. The tibial axis was approximately collateral with body axis (mean = 12.2 degrees difference) and in 80% of the tests it did not vary from parallel by more than 20 degrees. In the following account, the descriptions of the displacement direction relative to the tibia can therefore be taken as roughly equivalent to those relative to the body long axis.

To correlate afferent activities with the orientation of the animal, we first classed tests as lateral or parallel perturbations according to the direction of the platform displacement relative to the tibia. Lateral perturbations were those in which the substrate movement was perpendicular (± 45 degrees) to the long axis of the tibia (Fig. 14A), while the displacement was oriented collateral (± 45 degrees) to the tibial axis in parallel perturbations (Fig. 14D). We further defined the movement of the platform in lateral perturbations as being initiated from the contralateral or ipsilateral side, relative to the leg from which recordings were taken. Parallel perturbations were classed as moving from the head toward the abdomen or from the abdomen toward the head.

Figures 14B and 14C show examples of sensory responses during the early period of tests in which the arena moved in a direction lateral to the tibial axis. The proximal receptors fired a rapid, short latency burst when the displacement was initiated from the

Figure 14: Sensilla show directional responses during ramp perturbations. The tibial receptors fired regularly during ramp displacements and their activity was dependent on the direction of platform movement. **A** Lateral perturbations were defined as those trials in which the axis of the tibia was oriented perpendicular ($\pm 45^\circ$) to the direction of displacement. **B-C** Platform translations oriented laterally elicited excitation of the proximal sensilla when platform movement was directed from the contralateral side (**B**) while distal sensilla responded when translations were initiated from the ipsilateral side (**C**) (amplitude- 3.2 mm, rate- 55.5 mm/sec). The frequency histograms above the recordings plot the sensory activity (SENS1) in bins of 8 ms (PRX/DISTAL FREQ). The arrowheads designate the start of platform movement. **D** In parallel perturbations the tibia was oriented parallel ($\pm 45^\circ$) to the direction of platform movement. **E-F** Displacements directed parallel to the long axis of the tibia excited the proximal sensilla when movement was from the head towards the abdomen (**E**) while distal receptors were activated when the platform moved towards the head (**F**) (amplitude- 3.2 mm, rate- 55.5 mm/sec). In all diagrams, the left metathoracic leg (from which recordings were taken) is drawn in bold.



contralateral side (Fig. 14B), while the distal sensilla were activated by translations from the ipsilateral side (Fig. 14C). The sensilla also showed responses when the platform was displaced parallel to the body long axis (Figs. 14E,F). Proximal receptors were stimulated when the direction of movement was oriented from the head towards the abdomen while distal sensilla fired when the platform moved in the opposite direction. This directionality was invariant and co-activation of proximal and distal receptors within the first 30 ms did not occur.

A number of observations and controls confirmed that this early discharge was the result of horizontal displacement of the platform and was not the consequence of vibration in our apparatus. Receptor responses depended upon the orientation of the animal relative to the direction of displacement. However, they were not related to its distance from the coil, as could occur if the arena were displaced vertically as an unsupported beam. The differential responses of the campaniform sensilla could also be seen when the animal turned in place and altered the direction of displacement without changing its distance from the coil. In some experiments, we rotated the arena by 90 degrees so that the short axis was perpendicular to the direction of movement (data not shown). This effectively brought the animal closer to coil but had no effect upon sensillum discharges. Lastly, responses of the receptors showed thresholds that were dependent both upon the amplitude and velocity of platform displacement. These findings support the idea that the responses of the sensilla result from the forces that were applied to the legs during the translation and were not the consequence of substrate vibration.

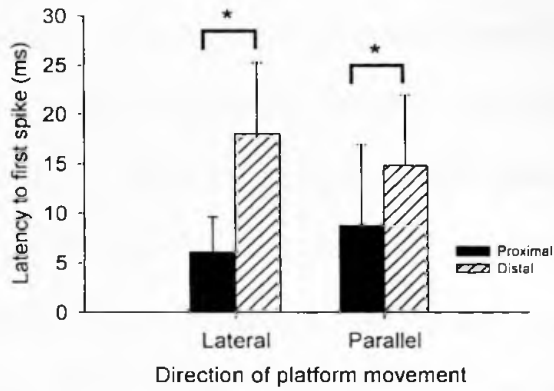
Threshold and response latencies

The proximal and distal campaniform sensilla differed in the rate and amplitude of displacement at which they became active and in the latency of their spiking activity from the onset of platform movement. Prior to movement of the platform, the proximal receptors were often tonically active at low levels (up to 40 Hz) in freely standing animals. In contrast, the distal sensilla rarely showed tonic activity. The proximal receptors had a low threshold of response in that they were activated at a lower rate of platform displacement than the distal sensilla (see rate sensitivity section).

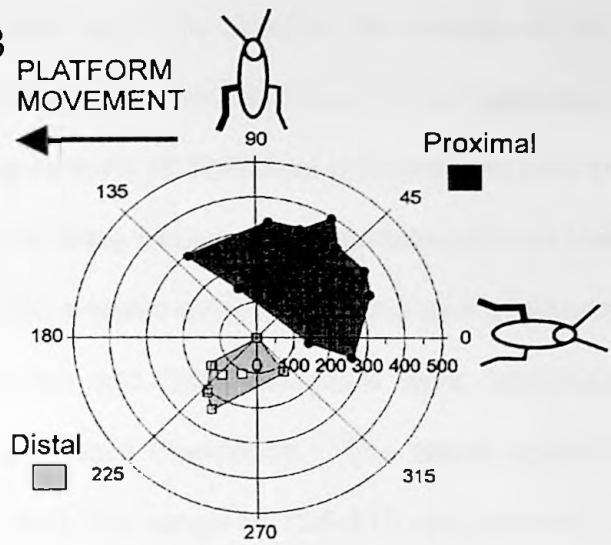
Response latencies of the tibial sensilla were calculated from the onset of the ramp perturbation to the first sensory spike. The receptors were rapidly activated and latencies to burst onset were extremely short. Response latencies of the proximal sensilla were less than the distal receptors in most ramp perturbations at the same rate. The graph in Figure 15A plots the mean latency of proximal and distal receptor activity during lateral and parallel perturbations. When tests in all directions and rates were analyzed and grouped, the proximal sensilla fired within 6.1 ± 3.5 ms (mean \pm standard deviation, $n = 61$) of platform movement during lateral perturbations and 8.8 ± 8.2 ms ($n = 49$) during parallel displacements. The distal receptors responded within 18.1 ± 7.2 ms ($n = 39$) of platform movement during lateral translations and 14.8 ± 7.1 ms ($n = 30$) during parallel perturbations. Although these grouped figures show variability, the response latencies of the proximal sensilla were significantly shorter than the distal receptors in both lateral and parallel perturbations (ANOVA, $p < 0.001$). However, the latencies of the tibial sensilla were similar when lateral and parallel translations were compared.

Figure 15: Response latencies and encoding of perturbation direction. **A** The latencies to onset of firing of the tibial campaniform sensilla are extremely short. The mean latencies from the beginning of platform movement to the first spike of proximal and distal sensilla are plotted for lateral (proximal n=61; distal n=39) and parallel (proximal n=49; distal n=30) perturbations. Error bars = standard deviation (SD). There is no significant difference in latencies between lateral and parallel perturbations but the onset to firing of the proximal sensilla is shorter than the distal receptors in both directions of movement (ANOVA, $p < 0.01$). **B-D** Polar plots of maximum firing frequencies of proximal and distal receptors and tibial orientation in three different animals (rate of platform movement = **B**- 43.3 mm/sec, **C**- 55.5 mm/sec, **D**- 35.7 mm/sec). In all trials examined, the proximal (closed circles) and distal sensilla (open squares) are activated in ranges of tibial orientation that are discrete and do not overlap. **E-F** Data from the three polar plots (B-D) were grouped (into 45 degree bins) and averaged in order to compare the responses of the sensilla at different tibial orientations. In the range of 315-0°, proximal receptor activity was significantly less when compared to 45-90° or 0-45° (**E**; ANOVA, $p < 0.05$; Fisher's Protected Least Significant Difference [PLSD], # = $p < 0.04$, * = $p < 0.02$). The distal sensilla also showed a significant decrease in activity in the range 135-180° when compared with 225-270° and 180-225° (**F**; ANOVA, $p < 0.05$; Fisher PLSD, # = $p < 0.02$, * = $p < 0.01$).

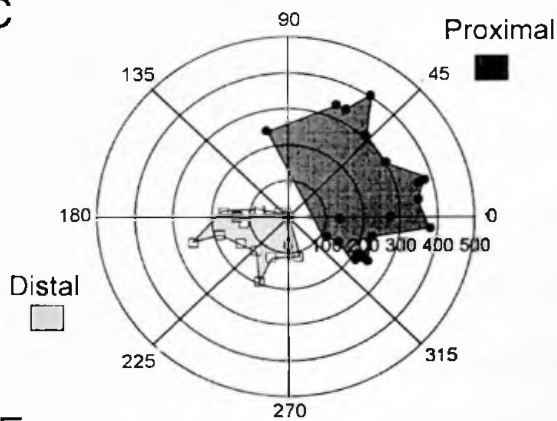
A



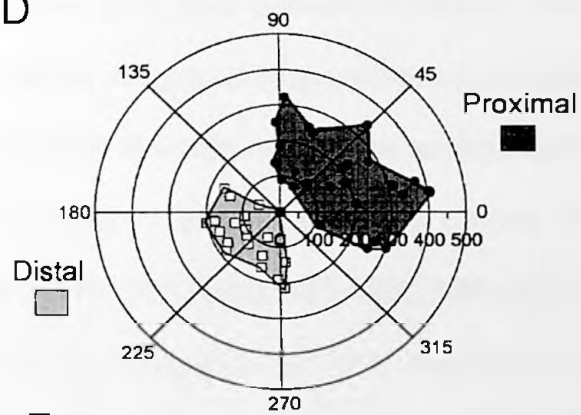
B



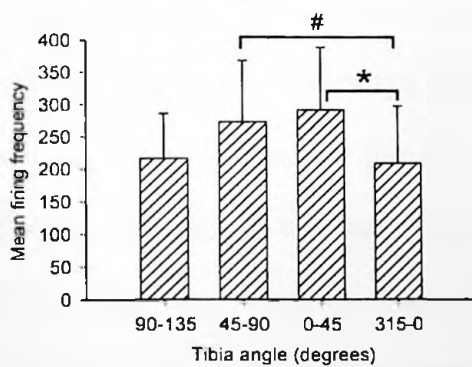
C



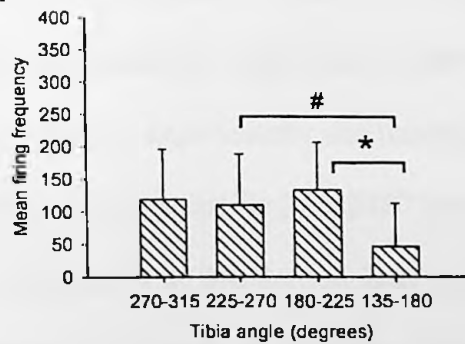
D



E



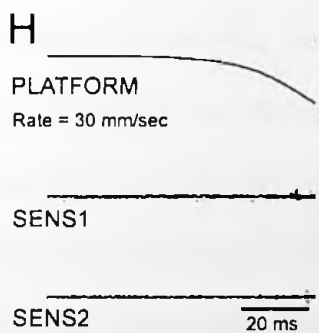
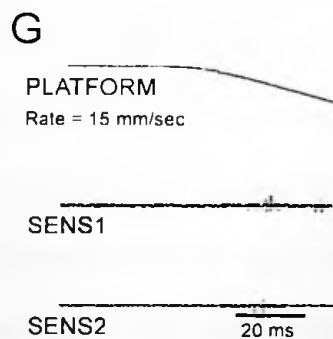
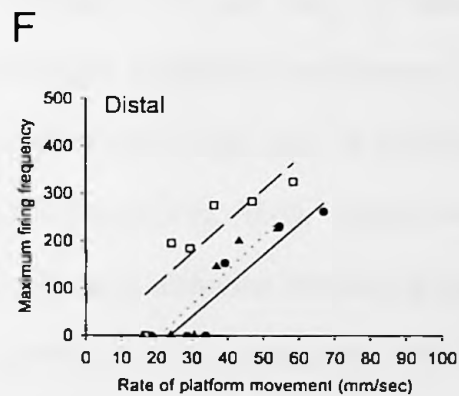
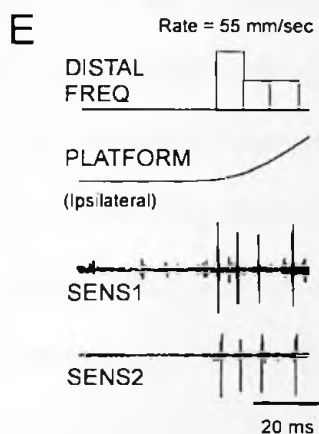
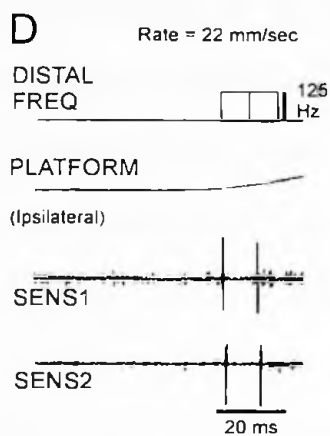
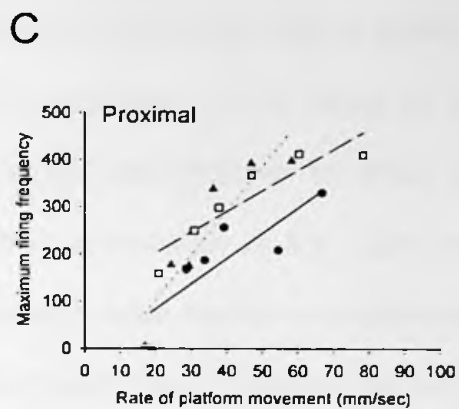
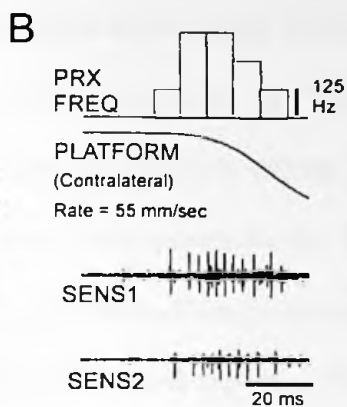
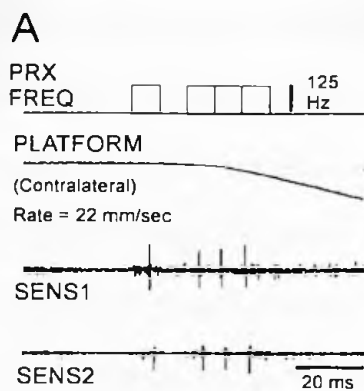
F



Directional sensitivities of the tibial sensilla

In order to characterize the directional sensitivity of the sensilla, the activity of the receptors relative to the direction of platform movement was examined. The responses of the sensilla showed consistent directionality in tests of individual preparations and in summed data. Figures 15B-D plot the maximum firing frequency of the receptors on the radial axis and the orientation of the tibia on the angular axis for all ramp perturbations in 3 animals. In each experiment, the proximal and distal receptors were activated within discrete, and non-overlapping, ranges of tibia orientation. The distal sensilla were stimulated when the leg was oriented within the range of 135-315 degrees while the proximal receptors were excited when the tibia was oriented between 315-135 degrees. Furthermore, the sensilla show similar ranges of responsiveness in each of the experiments, although the specific orientations at which tests were applied were not controlled. When the experiments were compared according to the ranges of leg angles tested (proximal, 315-0°, 0-45°, 45-90°, 90-135°; distal, 135-180°, 180-225°, 225-270°, 270-315°), significant differences in the magnitude of the responses were observed. In the proximal sensilla, the mean firing frequency (mean of the maximum) was significantly less in the range of 315-0° as compared to 0-45° and 45-90° (Fig. 15E, ANOVA, $p < 0.05$). Activity of the distal receptors also significantly decreased when the animal was oriented in the range of 135-180° as compared to 225-270° and 180-225° (Fig. 15F, ANOVA, $p < 0.05$). These findings suggest that the forces acting on the tibia are reduced in these ranges of orientation, although further studies are needed to fully characterize the activity of the receptors to all possible ranges of perturbation direction.

Figure 16: Campaniform sensilla discharges reflect the rate of displacement. **A-B** The proximal sensilla show an increase in firing frequency with more rapid platform movements. These recordings are examples of proximal sensilla activity when the platform moved at 22 mm/sec (**A**) and 55 mm/sec (**B**). The histograms above the raw recordings plot the firing frequency of the receptors in 8 ms bins (PRX/DISTAL FREQ). **C** This graph plots the maximum firing frequency of the proximal receptors for six ramp displacements at progressively increasing rates of movement. Data are shown for three tests from three animals. The lines are the best linear fit to the data points. The positive slopes indicate that the activity of the sensilla increases over the ranges of platform movement tested. (*circles and solid line, $r^2 = 0.73$, slope = 5.4; squares and dash line, $r^2 = 0.84$, slope = 4.4; triangle and dotted line, $r^2 = 0.85$, slope = 9.4*). **D-E** Activity of the distal sensilla during perturbations of 22 mm/sec (**D**) and 55 mm/sec (**E**). The distal receptors also showed increased firing frequencies when the platform was displaced more rapidly. **F** Distal receptor activity is also positively correlated with the rate of arena movement. The threshold for activation of the distal sensilla is higher than the proximal receptors (compare this plot with **C**). (*circles and solid line, $r^2 = 0.86$, slope = 6.5; squares and dash line, $r^2 = 0.76$, slope = 6.7; triangle and dotted line, $r^2 = 0.84$, slope = 7.2*). **G-H** After ablation of the tibial sensilla, no activity is recorded during displacements at any of the rates tested (**G**, rate = 15 mm/sec; **H**, rate = 30 mm/sec).



Sensilla response to the rate of perturbation

The proximal and distal sensilla were highly sensitive to changing rates of platform movement. To characterize this sensitivity, we have recorded the activities of the sensilla in freely standing animals within a series of six perturbations in which the amplitude of displacement was held constant but the rate of rise of the ramp was increased. The discharge frequencies of the proximal and distal receptors consistently increased when the platform moved more rapidly. The recordings in Figures 16A and B show examples of the activity of the proximal sensilla (first 30 ms) in lateral perturbations initiated from the contralateral side at two rates of platform movement. In this example, the activity of the proximal receptors tripled when the rate of platform translation increased from 22 mm/sec (Fig. 16A) to 55 mm/sec (Fig. 16B). Responses of the distal sensilla during lateral perturbations in the opposite direction showed a two-fold increase in the firing frequency with more rapid platform movements (Fig. 16D-22 mm/sec; Fig. 16E-55 mm/sec).

To characterize the receptor responses we plotted the maximum discharge frequencies attained during the first 30 ms versus the rate of platform movement of each ramp displacement. The maximum was chosen for the analysis because the firing frequency within a burst often accelerated up to a peak and then decreased uniformly (see Figs. 13B, 14B). Figures 16C and F plot the frequency of the proximal (Fig. 16C) and distal (Fig. 16F) receptors during single tests of six ramp perturbations in three animals. The activity of both types of sensilla increased as the platform moved more rapidly. These graphs also illustrate the differences in threshold between the proximal and distal receptors. In the majority of ramp perturbations (89%, $n = 90$ out of 102), the

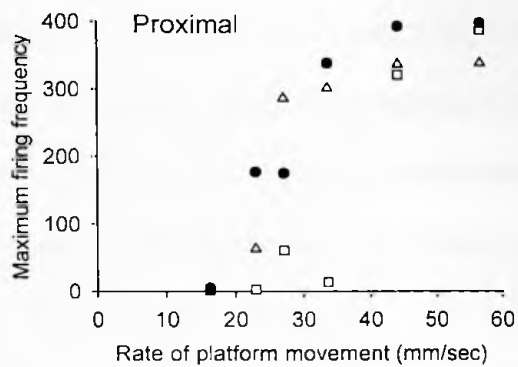
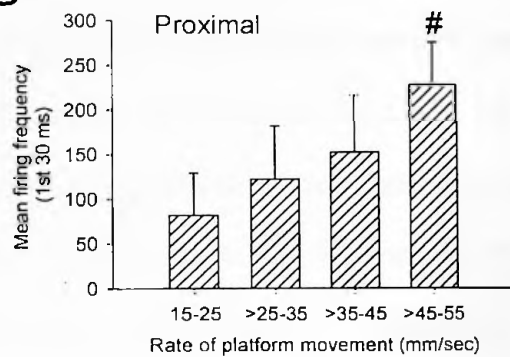
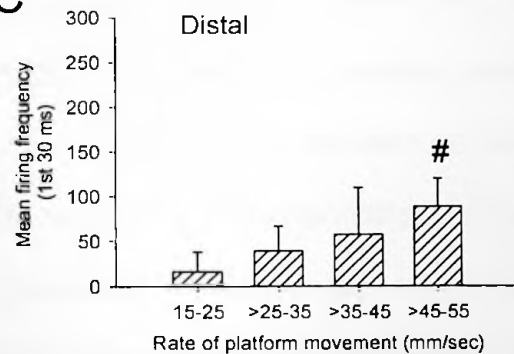
proximal receptors were activated during the slowest perturbation tests while the distal sensilla did not respond until the platform moved more rapidly. In the examples shown in Figures 16C and F, the proximal sensilla fired a burst of activity when the platform moved 28 mm/sec while the distal receptors were not stimulated until the platform was displaced at 39 mm/sec within the same test (compare filled circles).

To confirm that the tibial sensilla were responsible for the activity recorded during tests at variable rates of arena movement, the responses of the receptors were retested after cap ablation. Figures 16G and H show two recordings after the ablation during two rates of platform perturbations (Fig. 16G- 15 mm/sec, Fig. 16H- 30 mm/sec). The absence of responses during these platform movements confirms that the spikes recorded in these tests are derived from the tibial campaniform sensilla.

The sensilla showed considerable variability in the absolute value of their maximum discharge frequency during platform perturbations. However, the receptors can show similar discharge frequencies during tests in which the animal did not move and maintained its position for multiple repetitions. Figure 17A shows an example of proximal sensillum activity in a single preparation during three perturbation series in succession. This graph illustrates that the sensitivity of the receptors to the rate of platform movement can remain relatively constant if a single posture is maintained.

In order to test if the sensilla consistently discharged at higher rates to more rapid displacements, we calculated and averaged the mean firing frequencies of the receptors during the first 30 ms of lateral platform movements in a number of experiments (N= 32 tests in 5 animals). The responses of the proximal and distal receptors were plotted (Fig. 17B,C) against four ranges of arena velocity (15-25, >25-35, >35-45, >45-55

Figure 17: Receptor sensitivities to the rate of platform movement. **A** This graph plots the firing frequencies of the proximal sensilla for three sets of ramp displacements in a single animal that maintained its position throughout the series. The firing frequencies to changing rates of platform movement were similar when postures were maintained for long periods. **B-C** The mean firing frequencies of the proximal (**B**) and distal (**C**) sensilla during the first 30 ms of the perturbations were averaged and plotted for different ranges of platform movement (data from 5 animals). The activity of the proximal (ANOVA, $p < 0.01$; Fisher PLSD- all pair wise comparisons # = $p < 0.02$) and distal receptors (ANOVA, $p < 0.01$; Fisher PLSD- all pair wise comparisons # = $p < 0.02$) increased significantly at higher rates of platform movement .

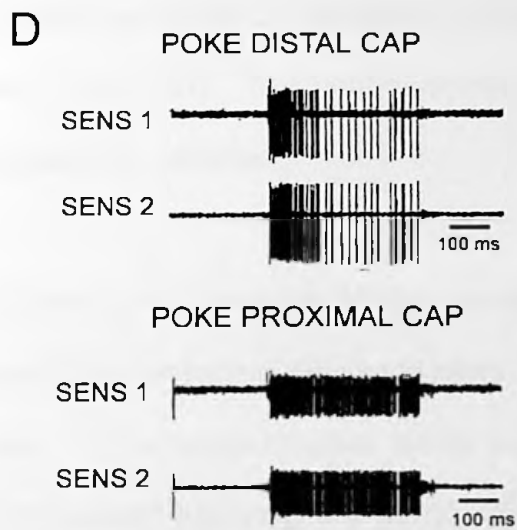
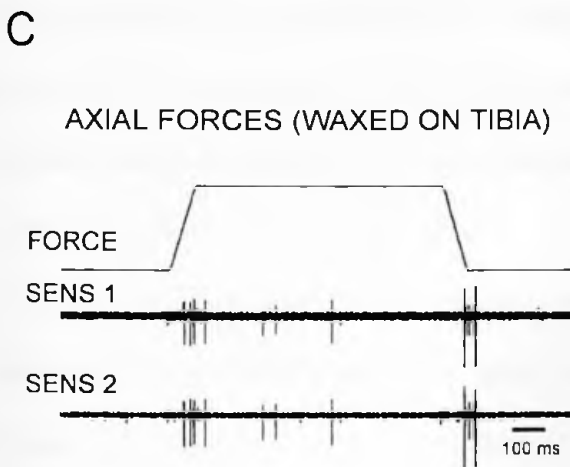
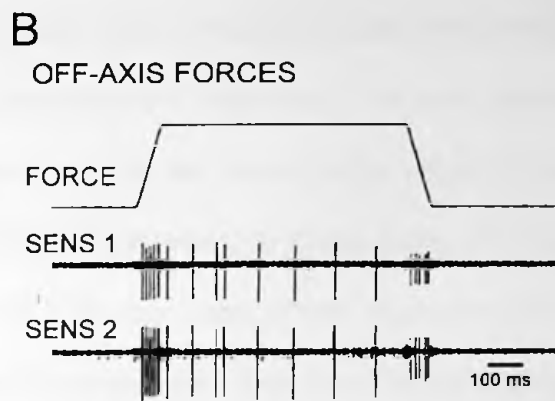
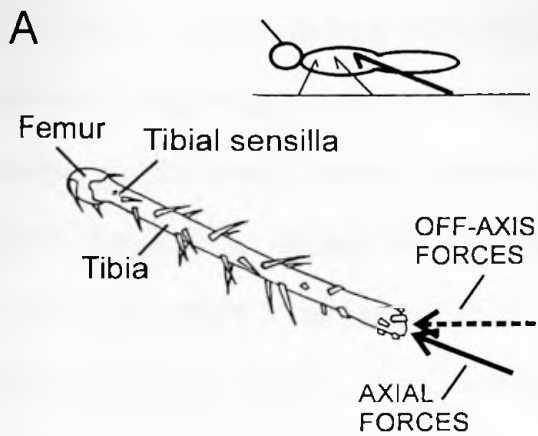
A**B****C**

mm/sec). Both the proximal and distal sensilla show a consistent and significant rise in activity as the rate of the perturbation increased (proximal, Fig. 17B, ANOVA, $p < 0.01$; distal, Fig. 17C, ANOVA, $p < 0.01$). These graphs further confirm that the firing frequency of the proximal receptors is higher than distal sensilla at similar rates of platform movement. Similar sensitivities to the velocity of substrate movement were also observed during tests in which the animal was oriented parallel to the direction of displacement (data not shown).

Responses to forces 'parallel' to the tibial axis: tests in restrained preparations

The tibial campaniform sensilla responded vigorously to perturbations directed parallel to the body long axis (Figs. 14, 15). This finding was unexpected, as forces directed along the tibial axis produce uniformly distributed compressions or tensions. Previous physiological (Zill and Moran 1981a) and modeling (Cocatre-Zilgien and Delcomyn 1999) studies in restrained preparations have shown that these strains generate lower discharge rates in the campaniform sensilla than bending perpendicular to the tibia. However, in a standing animal, the tibia of the hindleg is not strictly parallel with the substrate but is elevated on its proximal end so that its axis is raised between 15-25° relative to the horizontal plane (Larsen et al. 1997). This deviation could lead to the generation of both axial compressions/tensions and bending when forces are applied to the distal tibia via contact with the platform. To test this possibility, we applied forces in restrained preparations that were 'off axis', that is as compressions on the distal tibial which were angled relative to its axis (Fig. 18A). We also compared the responses of the sensilla in these experiments with tests in which forces were applied strictly axially, with a minimal bending component. Figure 18B shows an example of

Figure 18: Simulation of parallel perturbations **A** Forces were applied to the tibia in restrained animals to mimic the action of the platform movement during parallel perturbations. **B** Off axis forces. In standing cockroaches, the long axis of the tibia is at an angle of 15-20 degrees with the substrate. Forces applied to the tibia in this direction in restrained preparations (as compressions) activated the distal sensilla (similar to parallel perturbations oriented from the abdomen toward the head). **C** Axial forces. Forces were then applied to the leg directly along the tibia axis. The probe was waxed to the distal tibia to create a stiff linkage and minimize bending forces. The proximal receptors were excited when the force on the tibia increased, as shown in previous studies in restrained preparations. **D** Caps of individual sensilla were indented with a tungsten wire to identify the receptor units according to their spike amplitude.



distal receptor activity when a force probe was pressed against the distal end of the tibia at an angle of approximately 15° to its long axis. The force probe was then moved to a position perpendicular to the tibial axis and waxed to its distal end. The wax attachment created a mechanical linkage between the leg and the force probe which minimized lateral displacements that could generate bending forces. In these tests, the proximal sensilla fired upon force increases (Fig. 18C) as has been shown in previous studies (Zill and Moran 1981a). Individual receptor units were identified by indenting their cuticular caps with a tungsten wire probe, which produced a discharge of equivalent amplitude to that recorded during leg bending (Fig. 18D). This control confirmed that the response was derived from the tibial campaniform sensilla.

DISCUSSION

In the present study, we utilized horizontal displacements of the substrate to characterize the responses of the tibial campaniform sensilla in freely standing animals. These experiments have demonstrated that: 1) the sense organs show consistent discharges during the period (0-30 ms) immediately following the onset of platform movements; 2) the proximal and distal receptors respond to opposite directions of displacement during this interval; and 3) the firing frequencies of the tibial sensilla are highly sensitive to the rate of platform movement. These results are consistent with previous studies in freely moving animals (Zill and Moran 1981b, Zill et. al. 1981), which have shown that the tibial campaniform sensilla can encode forces in walking. In the following, we will first briefly review the abilities of insects to maintain stability during postural perturbations. We will then discuss the specific activities of the tibial

campaniform sensilla that occur in substrate displacements, in the context of the known response properties and directional sensitivities of the receptors.

Behaviors of animals during substrate displacements

Cockroaches readily maintained stable postures when the platform upon which they were standing was displaced. Insects have been shown to effectively maintain stability when perturbations are imposed during posture and walking in a number of previous studies (Bartling and Schmitz 2000; Cruse 1981a,b; Schmitz 1993). Substrate displacements have been applied to grasshoppers standing on the wall of an arena that was moved sinusoidally (Zill and Frazier 1990, Zill et al. 1992) and in experiments utilizing horizontal platform movements to perturb walking cockroaches (Zill 1993). Those experiments showed that animals were readily able to stand on surfaces that were displaced, even in the absence of visual inputs (Zill and Frazier 1990, Zill et al. 1992). Insects are considered to be relatively stable when standing because the area supported by the legs is large and the center of mass is near the ground (Full 1997). Several studies have also demonstrated that considerable forces are needed to dislodge insects due to the adherence of the tarsi to the substrate (Misener and Boiteau 1993; Jiao et al. 2000). Bartling and Schmitz (2000) have recently studied the responses of freely moving stick insects to horizontal substrate displacements, by using a small platform (containing a force plate) that permitted perturbation of only one leg when standing or walking. They found that forces were rapidly developed in the leg to counter the imposed perturbation and concluded that these responses were generated by muscle contractions through negative feedback reflexes from leg sense organs. In addition, several authors have suggested that legs contain elastic elements that can

serve as passive compliances in resisting postural perturbations (Blickhan and Full 1993; Frazier et al. 1999; Neff et al. 2000). In the present experiments, small displacements (see Fig. 13A) or those occurring at very low rates did not elicit sensory discharges or activities in the slow tibial extensor motoneuron, consistent with this hypothesis. Thus, the abilities of insects to resist perturbations is most likely due to both neuromuscular mechanisms and inherent features of their leg design.

Directional sensitivities of proximal and distal subgroups

The tibial campaniform sensilla are rapidly excited following the initiation of platform movement. Substrate displacements produce accelerations at their onset that exert forces upon the legs when the foot remains in contact with the moving surface (Jacobs and Macpherson 1996; Bartling and Schmitz 2000). We consider that the discharges of the campaniform sensilla result from forces that are similarly transmitted to the tibia, as they are correlated with the direction and velocity of platform movement and do not appear to be a result of vibration or other mechanical stimuli in our apparatus. In each position assumed by the animal, we applied ramp displacements that were paired, so that the platform first moved in one direction and then returned at the same rate and amplitude in the opposite direction. During the first 30 ms following the onset of translation, the proximal and distal sensilla fired to opposite directions of the paired movements. The exact forces and joint torques that were generated during substrate displacements are unknown. However, the discharges of the tibial sensilla are consistent with the directionality that has been demonstrated in restrained preparations to applied bending and axial forces (Spinola and Chapman 1975; Zill and Moran 1981a). Cocatre-Zilgien and Delcomyn (1999) have also recently successfully modeled

the responses of the tibial campaniform sensilla as directionally sensitive strain gauges. Thus, the studies to date support the idea that the proximal and distal subgroups encode strains that result from different and antagonist force vectors in both restrained and freely moving animals.

Encoding of forces in lateral and parallel perturbations

The tibial campaniform sensilla, as a group, responded over a broad range of displacement directions, including translations perpendicular to and parallel with the long axis of the tibia. During platform displacements, the forces that are exerted upon the legs depend upon their orientation with the substrate (Macpherson 1994). In the present study, measurements from video images showed that the tibia was frequently held parallel to the body long axis, with the femoro-tibial joint initially at an angle of approximately 90 degrees. The tibia was elevated on its proximal end from the horizontal by 10-20 degrees. In the following, we will present a hypothesis as to the mechanical forces that can generate the observed discharges of the tibial sensilla and compare them with results from forces exerted upon the leg in restrained preparations.

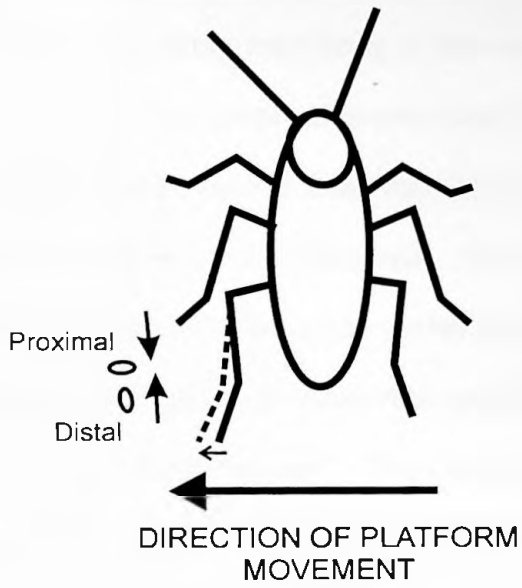
We propose that substrate translations produce a bending of the tibia as the foot initially moves with the platform (Fig. 19). In the orientation most frequently seen during perturbation experiments, lateral displacements would act to produce forced extensions and flexions of the joint. Displacements directed from the contralateral side would generate forces to produce transient extensions (Fig. 19A) while translations in the opposite direction would generate joint flexions. These findings are consistent with experiments in restrained cockroaches which have shown that the sensilla can be transiently activated by movements when the femoro-tibial joint is not immobilized (Zill

and Moran 1981a). Joint extensions produce activation of the proximal sensilla, while flexions excite the distal receptors. Data from some tests support this hypothesis in that small changes in leg joint angles in these directions were detected, coincident with activity of the tibial sensilla (Fig. 13).

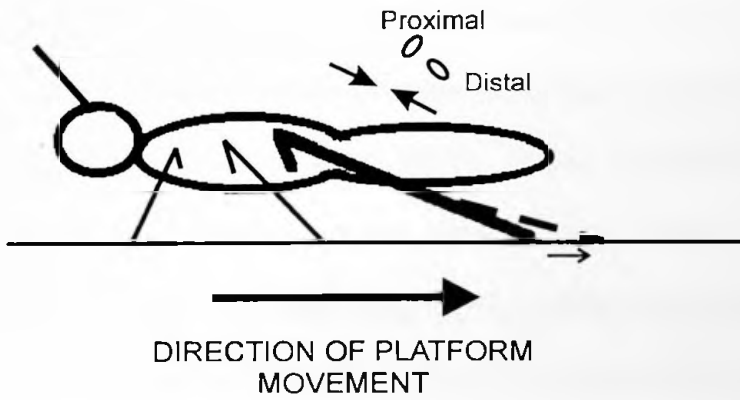
Perturbations directed parallel to the body long axis also excited the proximal and distal receptors (Figs. 14, 15). The forces that produce these types of discharges are less apparent. As we have noted, forces applied in this direction probably do not produce strict axial compressions or tensions, due to the angle of orientation of the tibia with the substrate ($15-25^{\circ}$ relative to the horizontal plane, Larsen et al. 1997). This deviation could lead to the generation of both axial compressions/tensions and bending when forces are applied to the distal tibia via contact of the tarsus with the platform. We suggest that transient bending forces are exerted upon the tibia as shown in Figure 19B. Displacements from the head towards the abdomen could bend the distal tibia dorsally, exciting the proximal receptors (Fig. 19B). Movements of the platform in the opposite direction (abdomen towards the head) could generate ventral bending and excite the distal receptors. Our tests in restrained preparations provide some support for these hypotheses. Compressions directed somewhat 'off-axis' produced excitation of the distal sensilla, instead of the activation of proximal receptors as is seen during strict axial compression. Further experiments, such as those using strain gauges attached to the tibia (Newland and Emptage 1996) to monitor leg bending, would help in understanding how these perturbations generate the observed pattern of receptor activation.

Figure 19: Model of sensilla activation during platform perturbations. In this model, movements of the substrate produce forces that are transmitted through the exoskeleton of the leg. These forces generate transient bending of the tibia, as the foot (tarsus) remains in contact with the platform (illustrated as dotted line). Substrate perturbations of different orientations produce bending in different directions. **A** Lateral displacements from the contralateral side of the body produced excitation of the proximal sensilla during platform perturbations. This could result if the perturbation generated dorsal bending of the tibia, which is known to excite the proximal receptors (see diagram). **B** Parallel perturbations directed from the head towards the abdomen. Displacements of this orientation also excited the proximal sensilla. Dorsal bending of the leg could occur in these perturbations, as the tibia is at an angle with the horizontal plane. The present study has shown that these 'off-axis' forces can produce excitation of the sensilla consistent with the results seen in platform displacement.

A MODEL OF LATERAL PERTURBATION



B MODEL OF PARALLEL PERTURBATION



Variability in sensory discharge frequencies

The absolute firing frequency of the receptors could show variability between tests, even when identical amplitudes and rates of displacement were compared. The goal of these experiments was to examine the responses of the tibial sensilla in preparations that were completely unrestrained. Cockroaches were, therefore, free to assume a variety of postures. While we monitored the position of the hindleg tibia from which sensory recordings were taken, the animal's other legs could show varied orientations relative to the displacement. The forces exerted by those legs would therefore differ among tests. Furthermore, we were unable to completely control for the possibility that the abdomen transiently rested against the substrate, providing further support. Variability in loading of a single leg could also result from the fact that a six-legged animal can maintain a stable posture using only three of its legs for support (e.g., *cerca* grooming, Reingold and Camhi 1977). While the data from force plate measurements in standing cockroaches are not available, the forces on single legs show continuous changes in cats during quiet stance (Horak and Macpherson 1996). Similar changes in the baseline levels of force exerted by the leg could sum with those imposed during the displacements and contribute to the observed variation in afferent discharges.

Variability in receptor firing could also be derived from muscle tensions, which are known to produce strains in the exoskeleton that can excite or inhibit the campaniform sensilla (Zill and Moran 1981a,b). While we monitored the activities of the tibial extensor muscle, its discharges were generally quite low in animals that were standing and not walking. However, we did not directly monitor the activities of the tibial flexor muscle. Tensions in the tibial flexor can produce activation of the proximal sensilla (Zill

et al. 1981) and potentially contribute to the variability we found in its background levels of firing. Lastly, a number of insect muscles are known to have considerable endogenous tonus that can be modulated in the absence of activation by excitatory motoneurons (Hoyle 1978). These types of changes in muscle tension could potentially contribute to the variability in the discharges we obtained to platform perturbations.

Encoding of rate of platform movement

A consistent finding in all tests was that both proximal and distal sensilla increased their firing to increasing rates of platform movement. Although the absolute firing frequencies of the sensilla varied among test series, the maximum and mean discharge rates were higher within single sequences at increasing ramp rates. Furthermore, when animals held positions over long periods, similar discharges could be obtained upon repetitions of series of ramps. These findings are consistent with results obtained in restrained preparations (Ridgel et al. 1999, 2000) and suggest that the phasic discharges of the sensilla indicate the rate of change of force produced at the point of contact of the tarsus and the substrate. However, the absolute firing frequency during perturbations can be affected by other variables, such as strains produced by muscle tensions or the extent of support provided by other legs.

The discharges we observed during platform displacements may also occur to changing forces during walking, if a leg slips. Slipping can produce both sudden decreases in forces in the leg that loses frictional contact and sudden increases in force in the remaining legs. The signals from the campaniform sensilla could readily provide indicators that these changes had occurred and information about the rate of change of forces.

Differences in responses of proximal and distal sensilla

The distal and proximal sensilla showed differences in their response properties in tests of postural perturbations. The distal receptors had higher thresholds and fired at lower frequencies than the proximal sensilla during tests at the same rate of platform movement. Similar differences in thresholds and rate sensitivities have been observed in restrained animals (Ridgel et al. 2000). We have suggested that these differences result from the structure and mechanical properties of the leg. For example, the distal sensilla are immediately adjacent to a large cuticular spine in an area associated with a high degree of apparent sclerotization (Andersen et al. 1996). Variability in cuticular thickness could also affect the nature of compressive strains acting on the tibia when forces are applied to the leg (Cocatre-Zilgien and Delcomyn 1999).

Sensory modalities activated during platform perturbations

In the present study, we found that discharges of the tibial campaniform sensilla occurred early in the ramp phase of platform displacements, often preceding activities in the tibial extensor muscle. This finding is important as compensatory motor reactions to substrate movements can be initiated at very short latencies in a number of systems (Horak and Macpherson 1996; cats, Macpherson 1988b; humans, Corna et al. 1999, Diener et al 1988, Dietz et al 1992, Nashner 1976, 1977, Nashner et al. 1979). These reactions are thought to be adjusted to the direction, velocity and amplitude of perturbations by information provided by sense organs that are active during the early phase of the displacement (Aniss et al. 1990; Diener et al. 1984; Dietz and Duysens 2000; Inglis et al. 1994). However, we also found that changes in joint angles frequently occurred during platform movements, as has been widely documented in other studies

(Bartling and Schmitz 2000). It is therefore likely that both receptors monitoring forces and those indicating changes in joint angles discharge during this period. Further studies are planned to examine how information on joint kinematics and forces are integrated into postural reactions in cockroaches. Our results suggest that substrate perturbations, that have been widely used to examine the control of posture and locomotion in vertebrates, may be utilized in future studies of insects to similar advantage.

GENERAL SUMMARY

The three chapters presented in this dissertation have examined the response properties of the tibial campaniform sensilla on the cockroach leg in restrained preparations and in freely moving animals during substrate perturbations. In this summary, we will review the major findings of these studies and present our ideas for future experiments in this field.

(1) The tibial sensilla are sensitive to increases and decreases in the magnitude of forces on the legs. Forces exerted by the legs, as measured by force plates, have been previously shown to increase during the stance phase and decrease prior to lifting of a leg in swing (Full and Tu 1990,1991). The tibial receptors may provide information about the phase of the step cycle and could be important in the timing of leg movement. However, forces on the legs have not been measured in conjunction with sensilla activity in freely moving cockroaches. A force plate could be used to measure load exerted by the legs on the ground. However, this technique does not provide information about forces acting on a single leg. Forces in the cuticle of an individual leg could be measured by affixing small strain gauges to the tibia. This technique would allow for simultaneous measures of receptor activity and leg forces in freely moving animals.

(2) Sensilla responses also encode the rate of change of increases and decreases in force in restrained preparations. The sensitivities of the tibial sensilla to the rate of change of force increases are maintained even in the presence of static loads. The weight of the body must be supported by the legs for an animal to stand above the surface. Therefore, these findings support the idea that changes in leg forces, which

vary about this static level, can be readily detected. The rate sensitivity of the tibial receptors in the presence of static loads could be tested in freely moving animals by applying weights during tests of platform perturbation.

(3) Individual tibial sensilla respond with consistent directionality to all magnitudes and rates of force. This directional sensitivity is maintained in restrained preparations and freely moving animals. The tibial receptors are unique in that they consist of two subgroups of sensilla with mutually perpendicular cuticular caps. Therefore, the group as a whole can detect forces from a variety of directions. These data support the hypothesis that the receptors provide accurate information about the direction of force vectors and their resultant cuticular strains in the leg. Information about force direction could provide feedback to the leg muscles that are important in maintaining balance and posture. Further studies in this field should examine the feedback pathways that are important in the generation of postural responses in insects. Previous works have shown that the activity of the tibial sensilla can effect the responses of the leg muscles (Zill and Moran 1981a,b). Therefore, an understanding of the interaction between sensory afferents and motor output could help to interpret the mechanisms involved in the maintenance of stable postures.

(4) The tibial sensilla increase their firing at increasing rates of platform movement in freely standing animals. The responsiveness of the receptors to the rate of the translations was maintained in all directions of perturbations. Dynamic responses of the campaniform sensilla could aid in detection of rapid changes in forces on the legs that may occur in walking over obstacles or during slipping. This hypothesis can be tested in freely moving cockroaches during walking and climbing. Recordings from sensory

afferents in these animals should yield further insight into the role of the tibial campaniform sensilla in posture and locomotion.

ABSTRACT

The ability to detect changes in forces is important for effective use of a leg in posture and locomotion. This thesis examines how forces are detected in the legs of cockroaches by tibial campaniform sensilla. Campaniform sensilla are mechanoreceptors that encode forces through ovoid cuticular caps embedded in the exoskeleton. The tibial sensilla are unique in that they consist of two subgroups with mutually perpendicular cap orientations.

We characterized the responses of the tibial receptors in restrained preparations by applying forces to the leg at controlled magnitudes and rates. The tibial sensilla, as a group, were sensitive to increasing and decreasing forces. Discharges of individual afferents depended upon the direction of force application and the orientation of the receptor's cuticular cap. Tonic discharges of the sensilla signaled the magnitude of force while the phasic activity accurately encoded the rate of force application. Sensitivities to changing rates of force were strictly preserved in the presence of a wide range of static loads. These discharges could be utilized to adapt posture and walking when animals stand upon or traverse irregular terrains. Discharges to decreasing forces indicate leg unloading during walking and could rapidly signal force decreases during slipping or loss of ground support.

We also tested the response properties of tibial sensilla in freely standing animals. The substrate upon which the animal stood was displaced horizontally using ramp and hold stimuli at varied rates. The receptors showed short latency responses that were initiated in the early period of platform movement. The activity of individual sensilla depended upon the direction of displacement and the orientation of their cuticular cap.

Receptors of different cap orientations responded to different directions of displacement, although the range of directional sensitivities was relatively broad. Afferent responses were extremely sensitive to the rate of platform movement. These results support the hypothesis that discharges of the receptors result from forces that are transmitted to the tibia when the foot initially moves with the substrate. These studies confirm the findings in restrained preparations and suggest that tibial sensilla are tuned to monitor changing forces that could occur during posture and locomotion.

REFERENCES

- Aidley DJ (1998) The physiology of excitable cells. 4th Edition. Cambridge University Press, Cambridge
- Alexander RM (1992) Exploring biomechanics: animals in motion. Scientific American Library, New York
- Andersen SO, Peter MG, Roepstorff P (1996) Cuticular sclerotization in insects. *Com Biochem Physiol* 113B(4):689-705
- Aniss AM, Diener HC, Hore J, Gandevia SC, Burke D (1990) Behavior of human muscle receptors when reliant on proprioceptive feedback during standing. *J Neurophysiol* 64(2):661-670
- Appenteng K, Prochazka A (1984) Tendon organ firing during active muscle lengthening in normal cats. *J Physiol (Lond)*:353:81-92
- Barth FG (1981) Strain detection in the arthropod exoskeleton. In: Laverack MS Cosens DJ (eds) *Sense Organs*. Blackie, Glasgow, pp. 112-141
- Barth FG, Bohnenberger J (1978) Lyriform slit sense organ: threshold and stimulus amplitude ranges in a multi-unit mechanoreceptor. *J Comp Physiol* 125:37-43
- Bartling C, Schmitz J (2000) Reaction to disturbances of a walking leg during stance. *J Exp Biol* 203:1211-1233
- Bässler U (1987) Timing and shaping influences on the motor output for walking in stick insects. *Biol Cybern* 55: 397- 401
- Bässler U, Rohrbacher J, Karg G, Breutel G (1991) Interruption of searching movements of partly restrained front legs of stick insects, a model situation for the start of a stance phase? *Biol Cybern* 65: 507-514
- Blickhan R, Barth FG (1985) Strains in the exoskeleton of spiders. *J Comp Physiol A* 157:115-147
- Blickhan R, Full RJ (1987) Locomotion energetics of the ghost crab: II. Mechanics of the center of mass during walking and running. *J Exp Biol* 130:155-174
- Blickhan R, Full RJ (1993) Similarity in multilegged locomotion: Bouncing like a monopod. *J Comp Physiol A* 173:509-517
- Bohnenberger J (1981) Matched transfer characteristics of single units in a compound slit sense organ. *J Comp Physiol* 142:391-402

- Burleigh AL, Horak FB (1996) Influence of instruction, prediction and afferent sensory information on the postural organization of step initiation. *J Neurophysiol* 75(4): 1619-1628
- Burleigh AL, Horak FB Malouin F (1994) Modification of postural responses and step initiation: evidence for goal-directed postural interactions. *J Neurophysiol* 72(6):2892-2902
- Burrows M (1996) *The Neurobiology of an Insect Brain*. Oxford, Oxford University Press
- Cavagna GA, Heglund NC, Taylor CR (1977) Mechanical work in terrestrial locomotion: Two basic mechanisms for minimizing energy expenditure. *Am J Physiol* 233:R243-R261
- Chapman KM (1975) Proprioceptive indentation of the campaniform sensilla of cockroach legs. *J Comp Physiol* 96: 257-272
- Chapman KM (1965) Campaniform sensilla on the tactile spines of the legs of the cockroach. *J Exp Biol* 42: 191-203
- Chapman KM, Duckrow RB (1975) Compliance and sensitivity of a mechanoreceptor in the insect exoskeleton. *J Comp Physiol* 100:251-268
- Chapman KM, Pankhurst JH (1967) Conduction velocities and their temperature coefficients in sensory nerve fibers of cockroach legs. *J Exp Biol* 46:63-84
- Chapman KM, Duckrow RB, Moran DT (1973) Form and role of deformation in excitation of an insect mechanoreceptor. *Nature* 244(5416):453-454
- Chapman KM, Mosinger JL, Duckrow RB (1979) The role of distributed viscoelastic coupling in sensory adaptation in an insect mechanoreceptor. *J Comp Physiol A* 131:1-12
- Chapman KM, Smith RS (1963) A linear transfer function underlying impulse frequency modulation in a cockroach mechanoreceptor. *Nature* 197:699-700
- Clarac F, Dando MR (1973) Tension receptor reflexes in the walking legs of a crab, *Cancer pagurus*. *Nature* 243:94-95
- Cocatre-Zilgien JH, Delcomyn F (1999) Modeling stress and strain in an insect leg for simulation of campaniform sensilla responses to external forces *Biol Cybern* 81:149-160
- Conway BA, Hultborn H, Kiehn O (1987) Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp Brain Res* 68:643-656

Corna S, Tarantola J, Nardone A, Giordana A, Schieppati, M (1999) Standing on a continuously moving platform: is body inertia counteracted or exploited? *Exp Brain Res* 124:331-341

Cruse H (1981a) Is the position of the femur-tibia joint under feedback control in the walking stick insect? I. Force measurements. *J Exp Biol* 92:87-95

Cruse H (1981b) Is the position of the femur-tibia joint under feedback control in the walking stick insect? II. Electrophysiological recordings. *J Exp Biol* 92:97-107

Davies P, Petit J, Scott JJA (1995) The dynamic response of Golgi tendon organs to tetanic contraction of in-series motor units. *Brain Res* 690:82-91

Delcomyn F (1991) Activity and directional sensitivity of leg campaniform sensilla in a stick insect. *J Comp Physiol A* 168:113-119

DiCaprio RA, Ridgel AL, Zill SN (1998) Modeling detection of changing forces in an insect leg: White noise analysis of cockroach tibial campaniform sensilla. *Neuroethology Abstr* 115

Dickinson MH (1990a) Linear and non-linear encoding properties of an identified mechanoreceptor on the fly wing measured with mechanical white-noise stimuli. *J Exp Biol* 151:219-244

Dickinson MH (1990b) Comparison of encoding properties of campaniform sensilla on the fly wing. *J Exp Biol* 151:245-261

Dickinson MH (1992) Directional sensitivity and mechanical coupling dynamics of campaniform sensilla during chord-wise deformations of the fly wing. *J Exp Biol* 169:221-233.

Diener HC, Horak FB, Nashner LM (1988) Influence of stimulus parameters on human postural responses. *J Neurophysiol* 59(6):1888-1905

Diener HC, Dichgans J, Bootz F, Bacher M (1984) Early stabilization of human posture after a sudden disturbance: influence of rate and amplitude of displacement. *Exp Brain Res* 56:126-134

Dietz V (1992) Human neuronal control of automatic functional movements: interaction between central programs and afferent input. *Physiol Rev* 72:33-69

Dietz V (1998) Evidence for a load receptor contribution to the control of posture and locomotion. *Neuro Sci Beh Rev* 22(4):495-499

- Dietz V, Duysens J (2000) Significance of load receptor input during locomotion: a review. *Gait and Posture* 11:102-110
- Dietz V, Gollhofer A, Kleiber M, Trippel M (1992) Regulation of bipedal stance: dependency on "load" receptors. *Exp Brain Res* 89:229-231
- Dunbar DC, Horak FB, Macpherson JM, Rushmer DS (1986) Neural control of quadrupedal and bipedal stance: implications for the evolution of erect posture. *Am J Phys Anthropol* 69:93-105
- Duysens J, Pearson KG (1980) Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res* 187(2):321-332
- Duysens J, Clarac F, Cruse H (2000) Load regulating mechanisms in gait and posture: comparative aspects. *Physiol Rev* 80:83-133
- Flannigan WC (1998) Finite element modeling of arthropod exoskeleton. Master's thesis, Case Western Reserve University
- Flannigan WC, Quinn RD, Zill SN (1998) Finite element analysis of strain distribution and encoding of forces in an insect leg. *Proc. of the Fifth International Congress of Neuroethology*, number 228.
- Frazier SF, Larsen GS, Neff D, Quimby L, Carney M, DiCaprio RA, Zill SN (1999) Elasticity and movements of the cockroach tarsus in walking. *J Comp Physiol A* 185:157-172
- French AS (1989) Two components of rapid sensory adaptation in a cockroach mechanoreceptor neuron. *J Neurophysiol* 62:768-777
- French AS (1992) Mechanotransduction. *Ann Rev Physiol* 54:135-52
- French AS, Torkkeli PH (1994) The time course of sensory adaptation in the cockroach tactile spine. *Neuroscience Letters* 178:147-150
- Fukami Y, Wilkinson RS (1977) Responses of isolated Golgi tendon organs of the cat. *J Physiol* 265:673-689.
- Full RJ (1997) Invertebrate locomotor systems. In: Dantzlen W (ed) *Handbook of Comparative Physiology*. Oxford University Press, New York, pp 853-930
- Full RJ, Tu MS (1990) Mechanics of six-legged runners. *J Exp Biol* 148:129-46
- Full RJ, Tu MS (1991) Mechanics of a rapid running insect: two-, four-, and six-legged locomotion. *J Exp Biol* 156:215-231

Full RJ, Blickhan R, Ting LH (1991) Leg design in hexapedal runners. *J Exp Biol* 158:369-90

Full RJ, Yamauchi A, Jindrich DL (1995) Maximum single leg force production: cockroaches righting on photoelastic gelatin. *J Exp Biol* 198:2441-2452

Fung J, Macpherson JM (1999) Attributes of quiet stance in the chronic spinal cat. *J Neurophysiol* 82:3056-3065

Goslow GE, Stauffer EK, Nemeth WC, Stuart DG (1973) The cat step cycle: responses of muscle spindle and tendon organs to passive stretch within the locomotor range. *Brain Res* 60:35-54

Grillner S (1975) Locomotion in vertebrates: central mechanisms and reflex interactions. *Physiol Rev* 55:247-304

Hanson JP, Redfern MS, Mazumdar M (1999) Predicting slips and falls considering required and available friction. *Ergonomics* 42(12):1619-1633

Hatsopoulos NG, Burrows M, Laurent G (1995) Hysteresis reduction in proprioception using presynaptic shunting inhibition. *J Neurophysiol*. 73:1031-1042

Hibbeler RC (1993) *Statics and Mechanics of Material*. MacMillan, New York

Hiebert GW, Pearson KG (1999) Contribution of sensory feedback to the generation of extensor activity during walking in the decerebrate cat. *J Neurophysiol* 81:758-770

Hiebert GW, Whelan PJ, Prochazka A, Pearson KG (1995) Suppression of the corrective response to loss of ground support by stimulation of extensor group I afferents. *J Neurophysiol* 73(1): 416-420

Hiebert GW, Whelan PJ, Prochazka A, Pearson KG (1996) Contributions of hindlimb flexor muscle afferents to the timing of phase transitions in the cat step cycle. *J Neurophysiol* 75:1126-1137

Hiebert GW, Gorassini MA, Jiang W, Prochazka A, Pearson KG (1994) Corrective responses to loss of ground support during walking. II. Comparison of intact and chronic spinal cats. *J Neurophysiol* 71(2): 611-622

Hietler WJ (1974) The locust jump: specializations of the metathoracic femoral-tibial joint. *J Comp Physiol* 89:93-104

Hietler WJ, Burrows M (1977) The locust jump: I The motor programme. *J Exp Biol* 66:203-219

- Hofmann T, Bässler U (1982) Anatomy and physiology of trochanteral campaniform sensilla in the stick insect, *Cuniculina impigra*. *Physiological Entomology* 7: 413-426
- Hofmann T, Bässler U (1986) Response characteristics of single trochanteral campaniform sensilla in the stick insect, *Cuniculina impigra*. *Physiological Entomology* 11: 17-21
- Horak FB, Macpherson JM (1996) Postural orientation and equilibrium. In: *Handbook of Physiology*. Sec. 12. Oxford University Press, New York, pp 255-292
- Houk JC (1979) Regulation of stiffness by skeletomotor reflexes. *A Rev Physiol* 41:99-114
- Houk J, Henneman E (1967) Responses of Golgi tendon organs to active contractions of the soleus muscle of the cat. *J Neurophysiol* 30:466-481
- Hoyle G (1978) Intrinsic rhythm and basic tonus in insect skeletal muscle. *J Exp Biol* 73:205-233
- Inglis JT, Macpherson JM (1995) Bilateral labyrinthectomy in the cat: effects on the postural response to translation. *J Neurophysiol* 73(3): 1181-1191
- Inglis JT, Horak FB, Shupert CL, Jones-Rycewicz C (1994) The importance of somatosensory information in the triggering and scaling automatic postural responses in humans. *Exp Brain Res* 101:159-164
- Jacobs R, Macpherson JM (1996) Two functional muscle groupings during postural equilibrium tasks in standing cats. *J Neurophysiol* 76(4): 2402-2411
- Jami L (1992) Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions. *Physiol Rev* 72(3):623-666
- Jami L, Petit J, Proske U, Zytnicki D (1985) Responses of tendon organs to unfused contractions of single motor units. *J Neurophysiol* 53(1):32-42
- Jiao Y, Gorb S, Scherge M (2000) Adhesion measured on the attachment pads of *Tettigonia viridissima* (Orthoptera, insecta). *J Exp Biol* 203:1887-1895
- Juusola M, French AS (1995) Transduction and adaptation in spider slit sense organ mechanoreceptors. *J Neurophysiol* 74(6):2513-2523
- Klärner D, Barnes WJP (1986) The cuticular stress detector (CSD2) of the crayfish: II activity during walking and influences on leg coordination. *J Exp Biol* 122:161-175
- Klärner D, Barth FG (1986) The cuticular stress detector (CSD2) of the crayfish: I physiological properties. *J Exp Biol* 122:149-159

- Larsen GS, Frazier SF, Zill SN (1997) The tarso-pretarsal chordotonal organ as an element in cockroach walking. *J Comp Physiol A* 180:683-700
- Leibrock CS, Marchand AR, Barnes WJP (1996a) Force dependent response reversal mediated by low- and high-threshold afferents from the same mechanoreceptor in a crayfish leg. *J Neurophysiol* 76:1540-1544
- Leibrock CS, Marchand AR, Barnes WJP, Clarac F (1996b) Synaptic connections of the cuticular stress detectors in crayfish: mono- and polysynaptic reflexes and the entrainment of fictive locomotion in an in-vitro preparation. *J Comp Physiol A* 178:711-725
- Libersat F, Zill S, Clarac F (1987a) Single-unit receptors and reflex effects of force-sensitive mechanoreceptors of the dactyl of the crab. *J Neurophysiol* 57(5):1601-1617
- Libersat F, Zill S, Clarac F (1987b) Force sensitive mechanoreceptors of the dactyl of the crab: single-unit responses during walking and evaluation of function. *J Neurophysiol* 57(5):1618-1636
- Loeb GE (1981) Somatosensory input to the spinal cord during normal walking *Can J Physiol Pharmacol* 59:627-635
- Macpherson JM (1988a) Strategies that simplify the control of quadrupedal stance. I. Forces at the ground. *J Neurophysiol* 60(1): 204-217
- Macpherson JM (1988b) Strategies that simplify the control of quadrupedal stance. II. Electromyographic activity. *J Neurophysiol* 60(1):218-31
- Macpherson J M (1994) Changes in a postural strategy with inter-paw distance. *J Neurophysiol* 71: 931-40
- Macpherson JM, Fung J (1999) Weight support and balance during perturbed stance in the chronic spinal cat. *J Neurophysiol* 82:3066-3081
- Macpherson JM, Inglis JT (1993) Stance and balance following bilateral labyrinthectomy. *Prog Brain Res* 97:219-28
- Macpherson JM, Horak FB, Dunbar DC, Dow RS (1989) Stance dependence of automatic postural adjustments in humans. *Exp Brain Res* 78:557-66
- Malbert CH, Leitner LM (1993) Mechanoreceptors sensitive to flow at the gastroduodenal junction of the cat. *Amer J Physiol* 265:310-313

Maki BE, Whitelaw RS, Mcilroy WE (1993) Does frontal-plane asymmetry in compensatory postural responses represent preparation for stepping? *Neurosci Lett* 149(1):87-90

Mann DW, Chapman KM (1975) Component mechanisms of sensitivity and adaptation in an insect mechanoreceptor. *Brain Res* 97:331-336

Marchand AR, Liebrock CS, Auriac MC, Barnes WJP, Clarac F (1995) Morphology, physiology and in vivo activity of cuticular stress detector afferents in crayfish. *J Comp Physiol A* 176: 404-424

Matheson T, Field LH (1995) An elaborate tension receptor system highlights sensory complexity in the hindleg of the locust. *J Exp Biol* 198:1673-1689

McCrea DA (1998) Neuronal basis of afferent-evoked enhancement of locomotor activity. *Ann NY Acad Sci* 860:216-225

Mcilroy WE, Maki BE (1993) Do anticipatory postural adjustments precede compensatory stepping reactions evoked by perturbation? *Neurosci Lett* 164(1-2):199-202

Mcilroy WE, Maki BE (1994) The 'deceleration response' to transient perturbation of upright stance. *Neurosci Lett* 175(1-2):13-6

McVay EJ, Redfern MS (1994) Rampway safety: forces as a function of rampway angle. *Am Ind Hyg Assoc J* 55(7):626-634

Misener GC, Boiteau G (1993) Holding capability of the Colorado potato beetle to potato leaves and plastic surfaces. *Canadian Agricultural Engineering* 35(1):27-31

Mittlestaedt, H (1998) Origin and processing of postural information. *Neuro Sci Beh Rev* 22(4):473-478

Moran DT, Rowley JC (1975) High voltage and scanning microscopy of site of stimulus reception of an insect mechanoreceptor. *J Ultra Res* 50:38-46

Moran DT, Chapman KM, Ellis RA (1971) The fine structure of cockroach campaniform sensilla. *J Cell Biol* 48(1):155-73

Nashner LM (1976) Adapting reflexes controlling human posture. *Exp Brain Res* 26:59-72

Nashner LM (1977) Fixed patterns of rapid postural responses among leg muscles during stance. *Exp Brain Res* 30:13-24

- Nashner LM, Woollacott M, Tuma G (1979) Organization of rapid responses to postural and locomotor-like perturbations of standing man. *Exp Brain Res* 36:463-476
- Neff D, Frazier SF, Quimby L, Wang R-T, Zill S (2000) Identification of resilin in the leg of cockroach, *Periplaneta americana*: confirmed by a simple method using pH dependence of UV fluorescence. *Arthropod Structure and Development* 29:75-83
- Nelson GM, Quinn RD (1998) Posture Control of a Cockroach-like Robot. Proceedings of the 1998 IEEE International Conference on Robotics and Automation
- Nelson GM, Quinn RD, Bachmann RJ, Flannigan WC, Ritzmann RE, Watson JT (1997) Design and Simulation of a Cockroach-like Hexapod Robot. Proceedings of the 1997 IEEE International Conference on Robotics and Automation
- Newland PL, Emptage NJ (1996) The central connection and actions during walking of tibial campaniform sensilla in the locust. *J Comp Physiol A* 178: 749-762
- Nijenhuis ED, Dresden D (1956) On the topographical anatomy of the nervous system of the mesothoracic leg of the American cockroach, *Periplaneta americana*. *Proc K Ned Akad Wet C* 58:121-136
- Pearson KG, Collins DF (1993) Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. *J Neurophysiol* 70(3):1009-1017
- Pearson KG, Ramirez JM, Jiang W (1992) Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. *Exp Brain Res* 90:557-566
- Pearson KG, Misiaszek JE, Fouad K (1998) Enhancement and resetting of locomotor activity by muscle afferents. In: Kiehn O, Harris-Warrick RM, Jordon LM, Hultborn H, N. Kudo (eds) *Neuronal Mechanisms for Generating Locomotor Activity: Annals of the New York Academy of Sciences Volume 860*. New York Academy of Sciences, New York, pp 203-215
- Pringle JWS (1938a) Proprioception in insects. I A new type of mechanical receptors from the palps of the cockroach. *J Exp Biol* 15:101-113
- Pringle JWS (1938b) Proprioception in insects. II The action of the campaniform sensilla on the legs. *J Exp Biol* 15: 114-131
- Pringle JWS (1961) Proprioception in arthropods. In: Ramsay JA, Wigglesworth, VB (eds) *The Cell and the Organism*. Cambridge University Press, London, pp 256-282
- Prochazka A (1996) Proprioceptive feedback and equilibrium. In: *Handbook of Physiology*. Sec. 12. Oxford University Press, New York, pp 89-128

Prochazka A, Gorassini M (1998a) Models of ensemble firing of muscle spindle afferents recorded during normal locomotion in cats. *J Physiol* 507.1:277-291

Prochazka A, Gorassini M (1998b) Ensemble firing of muscle afferents recorded during normal locomotion in cats. *J Physiol* 507.1:293-304

Prochazka A, Wand A (1980) Tendon organ discharge during voluntary movement in the cat *J Physiol (Lond)* 303:385-390

Ramasubramanian S, Flannigan WC, Nelson G, Quinn R and Zill SN (1999) Modeling of load sensing during the stance phase of cockroach walking. In: Virk GS, Randall M, Howard D (eds) *Climbing and Walking Robots*. Univ. of Portsmouth, Professional Engineering Publishing Ltd, London Pp. 17-28

Reingold S, Camhi JM (1977) A quantitative analysis of rhythmic leg movements during three different behaviors in the cockroach, *Periplaneta americana*. *J Insect Physiol* 23:1407-1420

Ridgel AL, Frazier SF, DiCaprio RA, Zill SN (1999) Active signaling of leg loading and unloading in the cockroach. *J Neurophysiol* 81(3):1432-1437

Ridgel AL, Frazier SF, DiCaprio RA, Zill SN (2000) Encoding of forces by cockroach tibial campaniform sensilla: implications in dynamic control of posture and locomotion. *J Comp Physiol A* 186 (4):359-374

Schmidt M, Gnatzy W (1984) Are the funnel-canal organs the 'campaniform sensilla' of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? *Cell Tissue Res* 237:81-93

Schmitz J (1993) Load-compensating reactions in the proximal leg joints of stick insects during standing and walking. *J Exp Biol* 183: 15-33

Schmitz J, Bartling C, Brunn DE, Cruse H, Dean J, Kindermann T, Schumm M, Wagner H (1995) Adaptive properties of "hard-wired" neuronal systems. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 88: 165-179

Schnorbus H (1971) Die subgenualen sinnesorgane von *Periplaneta americana*: histologie und vibrationsschwellen. *Z Vergl Physiologie* 71:14-48

Segundo JP, Stiber M, Vibert JF, Hanneton S (1995) Periodically modulated inhibition and its postsynaptic consequences: II. Influence of modulation slope, depth, range, noise and of postsynaptic natural discharges. *Neuroscience* 68(3):396-719

Serway RA (1996) *Physics for scientists and engineers*. Volume I, 4th ed. Philadelphia, Saunders College Publishing