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Proprioceptors monitoring forces in a locust hind leg during kicking form negative feedback loops with flexor tibiae motor neurons

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Summary

In preparation for jumping and kicking, a locust slowly generates large forces in the femoral muscles of its hind legs and stores them in elastic distortions of the tendons and femoral cuticle. At the femoro–tibial joints, the semi-lunar processes are bent, the cuticle of the dorsal distal femur is crumpled, and the femur is expanded in a medio-lateral direction. We have analysed whether these distortions are monitored by sense organs and whether the information they provide is used to limit the forces generated and thus prevent structural damage to the joint.

The two sensory neurons comprising the lump receptor lie in a groove in the ventral part of the distal femur. The sensory neurons spike if force is applied to the flexor tendon when the joint is fully flexed, but not when it is extended. They also spike as the tendon of the flexor muscle slides into the ventral femoral groove when the tibia is fully flexed during the co-contraction phase of kicking. Their spike frequency correlates with the extent of bending of a semi-lunar process that provides a quantifiable measure of the joint distortions. If the tibia is not fully flexed, however, then muscle contractions still cause distortions of the joint but these are not signalled by sensory spikes from the lump receptor. The lump receptor, therefore, does not respond primarily to the joint distortions but to the movements or force in the flexor tendon.

Contractions of the flexor tibiae muscle caused by spikes in individual flexor motor neurons can evoke spikes in sensory neurons from the lump receptor when the joint is fully flexed. In turn, the sensory neurons cause a hyperpolarisation in particular flexor motor neurons in a polysynaptic negative feedback loop. The lump receptor could, therefore, regulate the output of the flexor motor neurons and, thus, limit the amount of force generated during co-contraction. It may also contribute to the inhibition of the flexors at the end of co-contraction that allows rapid kicking movements to occur.

Key words: joint, joint receptor, motor neuron, sensory feedback, locust, *Schistocerca gregaria*.

Introduction

Proprioceptors in legs may signal the movements and positions of joints, while also monitoring the force generated by the muscles and the strains in the skeleton. Most joints are equipped with an array of receptors that often appear to provide overlapping signals to the central nervous system. For example, in vertebrates, full proprioceptive sensitivity at a joint depends upon the combined actions of joint receptors, muscle receptors and cutaneous mechanoreceptors. In crustaceans, muscle receptor organs, chordotonal organs, cuticular stress detectors and tension receptors on muscle apodemes may all act in parallel (Mill, 1976). Similarly in insects, chordotonal organs, myochordotonal organs, joint receptors, cuticular strain detectors and tension receptors can be present (Braunig et al., 1981; Field and Matheson, 1998; Theophilidis and Burns, 1979) and may be supplemented by aggregations of exteroceptors into hair plates that also act as proprioceptors (Pringle, 1938; Wendler, 1964). Unravelling the contribution of a particular proprioceptor in the context of signalling by the others responding to the same stimuli is essential for understanding the way that movement is controlled. Combined feedback from the different proprioceptors can provide one of the ways that different motor patterns are executed by the same sets of motor neurons and muscles, but an individual proprioceptor can contribute specifically to the output of certain sets of motor neurons.

The hind legs of a locust have proved to be a useful model in which to examine the interplay between the motor commands, the mechanics of the joints and muscles, and sensory feedback (Burrows, 1996). These legs are used in walking but are specialised for powerful jumping and kicking movements. The motor pattern for these movements consists of three phases (Burrows, 1995; Godden, 1975; Heitler and Burrows, 1977a): first, an initial cocking phase, in which the tibia is fully flexed about the femur; second, co-contraction of

flexor and extensor tibiae muscles; third, a triggering phase, in which inhibition of the flexor motor neurons enables a rapid and powerful tibial extension to occur. The force required for these movements is generated by almost isometric co-contractions of the large extensor and the smaller flexor tibiae muscles once a tibia is locked in a fully flexed position. The muscular force generated during co-contraction bends the tips of the semi-lunar processes at the femoro–tibial joints and distorts the distal femur (Burrows and Morris, 2001), storing approximately half of the energy required for rapid extension of the tibiae (Bennet-Clark, 1975).

Many proprioceptive sense organs provide feedback to control and modify the output of the motor neurons used in this motor pattern. Campaniform sensilla signal the forces in the cuticle and a single-celled tension receptor monitors the force in a distal bundle of the flexor tibiae muscle (Matheson and Field, 1995). The movements and positions of the femoro–tibial joint are signalled by a chordotonal organ containing some 90 sensory neurons (Field and Burrows, 1982; Matheson and Field, 1990; Usherwood et al., 1968), a single strand receptor neuron (Braunig, 1985) and five joint receptor neurons (Coillot and Boistel, 1969; Heitler and Burrows, 1977b). We have sought to determine whether particular proprioceptors at this joint monitor the distortions of the distal femur and bending of the semi-lunar processes that occur during jumping and kicking but not during walking, climbing and other locomotory movements. At all stages in the moulting cycle, the co-contraction phase of the motor pattern can cause irreparable damage (Norman, 1995), but limiting the production of excessive muscular force might be especially important for recently moulted animals in which the cuticle will not have fully hardened. The frequency with which kicks can be elicited falls before and after a moult and newly moulted animals are unable to generate the characteristic motor pattern (Norman, 1996, 1997).

We have focussed on two of the joint receptor neurons called collectively ‘the lump receptor’; the three remaining joint receptors respond to extension of the femoro–tibial joint beyond 80° (Coillot, 1974). These two lump receptor neurons lie in a groove between the posterior wall of the femur and the ventral invagination, or lump in the ventral femur (Heitler and Burrows, 1977b). The posterior arm of the tendon of the flexor tibiae muscle slides into this groove when the tibia is fully flexed before a kick or a jump and rests directly on the receptors. The two sensory neurons respond to movement or force applied to the flexor tendon only when the femoro–tibial joint is in the fully flexed position (Heitler and Burrows, 1977b). Although cutting the nerve containing the axons of these receptors does not influence the co-contraction of flexor and extensor muscles, it does reduce the occurrence of kicking or jumping (Heitler and Burrows, 1977b; Jellema and Heitler, 1997; Jellema et al., 1997).

To determine the possible functional roles of the lump receptor in monitoring events during kicking, we recorded its activity and the distortion of the semi-lunar processes. We analysed whether individual flexor motor neurons could differentially activate the lump receptor through their particular innervation patterns of the flexor muscle and the different forces they generate at the flexor tendon. Finally, we determined whether sensory neurons of the lump receptor make feedback loops with flexor motor neurons, building on the single example of such an effect reported by Heitler and Burrows (1977a,b).

Materials and methods

Mature, adult, gregarious phase locusts, *Schistocerca gregaria* Forskål, of either sex were taken from our colony in the Department of Zoology, University of Cambridge. A locust was mounted, ventral surface uppermost, in Plasticine with the femur of its left hind leg fixed but with its tibia and tarsus free to move. A small window was cut into the ventral posterior surface of the distal femur of this hind leg to expose the lateral nerve that contains the axons of the sensory neurons from the lump receptor. It was hooked onto a pair of 50 μm stainless steel electrodes. This nerve is purely sensory and contains the axons from the two sensory neurons of the lump receptor, from the three joint receptors activated by extension movements of the femoro–tibial joint, and from hairs on the distal posterior part of the femur (Heitler and Burrows, 1977b; Siegler and Burrows, 1983). A second pair of the same-sized wires was inserted into the extensor tibiae muscle to monitor its activity during kicking, or to stimulate electrically the axon terminals of its motor neurons, and two further pairs into the proximal and distal parts of the flexor tibiae muscle. All the recordings were sampled at 5 kHz and written directly to a computer with a CED (Cambridge Electronic Design, Cambridge, UK) interface running Spike2 software. Some nerve recordings were electronically filtered.

Images of the femoro–tibial joint during a kick, or during direct electrical stimulation of the extensor tibiae muscle were captured with a high speed camera (Redlake Imaging, San Diego, California, USA) and associated computer at 1000 frames s^{-1} and with an exposure time of 0.5 ms. Selected images were stored as computer files for later analysis with Motionscope software (Redlake Imaging). Images shown in the figures were timed from the point (0 ms) when the tibia reached full extension. Movements of the distal tip of a semi-lunar process at the femoro–tibial joint were measured from these images relative to a fixed point on the ventral femur. Video images on one computer were synchronized with the electrical recordings on a second computer, by generating 1 ms pulses from a hand-held switch when a kick was observed. These pulses were recorded as electrical events with the electrophysiological data and as light signals on the images.

Intracellular recordings were made in the metathoracic ganglion from the cell bodies of motor neurons innervating the flexor tibiae muscle of the left hind leg. The ganglion was exposed by removing the ventral cuticle of the thorax and then stabilized on a wax-coated silver platform. The thorax was perfused continuously with saline (Usherwood and Grundfest, 1965) at 20–22°C. The sheath of the metathoracic

ganglion was treated with protease (Sigma type XIV) for 30 s to facilitate the penetration of glass microelectrodes filled with 2 mol l^{-1} potassium acetate and with resistances of 40–80 MΩ. The flexor motor neurons were identified by the following criteria. First, the presence of a monosynaptic excitatory postsynaptic potential (EPSP) caused by an antidromic spike in the fast extensor tibiae (FETi) motor neuron (Burrows et al., 1989). Second, spikes evoked in the impaled flexor motor neuron by pulses of depolarizing current caused spikes that evoked flexion movements of the tibiae and could be matched with muscle potentials recorded extracellularly from flexor tibiae muscle bundles. Within the pool of flexor tibiae motor neurons, individuals could be classified as slow or fast-like, according to their threshold for spike initiation when depolarizing current was injected, by the frequency of evoked spikes and by the tibial movement they evoked.

To exert force on the tendon of the flexor tibiae muscle, it was exposed in some experiments by removing the ventral cuticle of femur in the left hind leg. After removal of the first and second proximal bundles of muscle fibres (Sasaki and Burrows, 1998), the tendon was grasped with fine forceps attached to a vibrator (Ling Dynamic, type 101). The tendon could then be moved linearly to mimic flexion movements of the femoro–tibial joint. The two main leg nerves, N5B1 and N5B2, were cut in the middle of the femur to remove inputs from mechanosensory neurons distal to the cut, except those innervated by the lateral nerve. The tendon of the extensor tibiae muscle was also cut at the same level so that extensor contractions could not cause sensory feedback.

The results are based on recordings from 52 locusts. 15 kicks by four locusts and 24 electrical stimulations of the extensor tibiae muscle in seven locusts were recorded with high-speed images. Intracellular recordings were made from 32 flexor motor neurons with simultaneous extracellular recordings from the lateral nerve in 20 locusts.

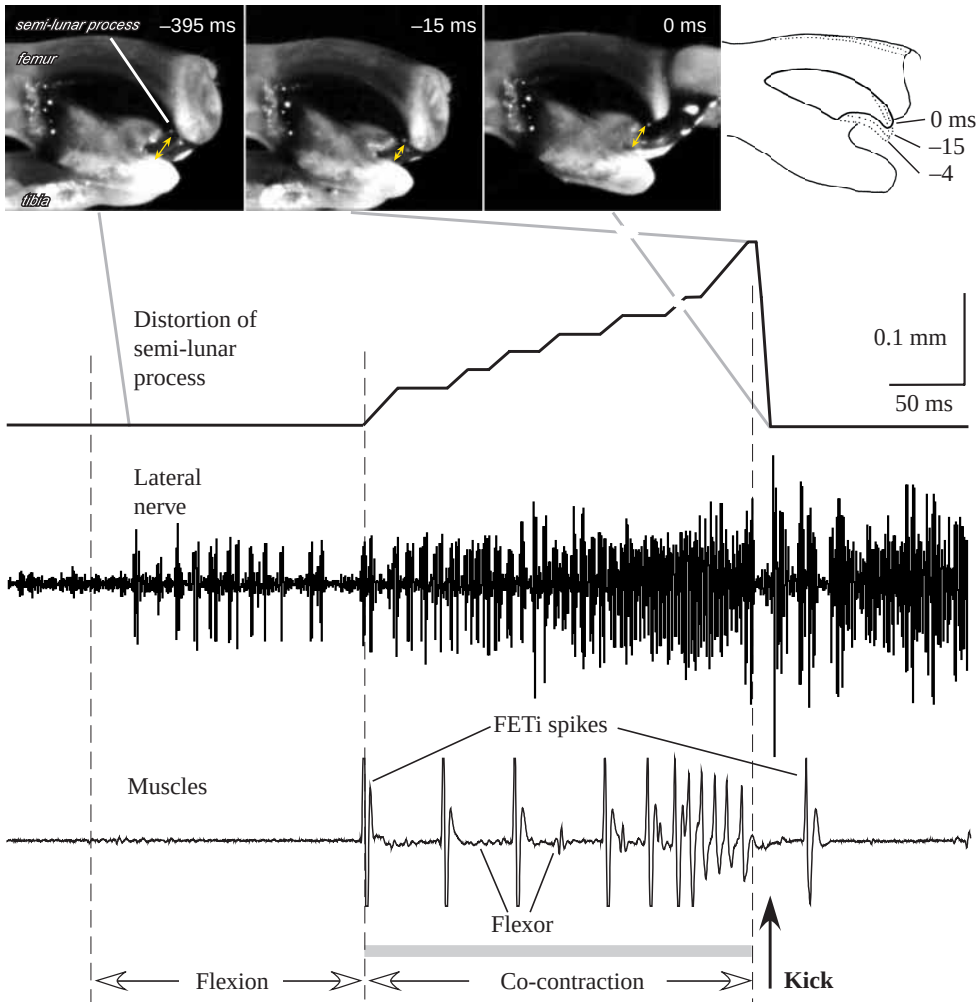

Fig. 1. Sensory signals from the lump receptor and distortion of the femoro–tibial joint during a kick. Electrical activity of the lateral nerve containing the axons of sensory neurons from the lump receptor, and of the extensor tibiae muscle was recorded at the same time as images of the movements of the femoro–tibial joint. The distortion of the lateral semi-lunar process was plotted from these images, three of which are shown at the times indicated. Full extension of the tibia in the kick occurred at time 0 ms. The arrows show the changing position of the distal tip of the semi-lunar process. The distortion of the dorsal femur and the bending of the semi-lunar process are also shown in tracings from frames at –15, –4 and 0 ms. The movements of the semi-lunar process in the graph appear jerky because of the intermittent sampling. Initial flexion of the tibia was accompanied by spikes in the lateral nerve. During the co-contraction phase (horizontal grey bar), the sensory spikes of the lump receptor occurred at high frequency as the semi-lunar process was bent progressively. The extended position of the tibia following the kick was signalled by sensory spikes in joint receptors. The vertical dashed lines indicate the different phases of the kick. The large spikes in the muscle recording are from the fast extensor tibiae motor neuron (FETi) and the smaller ones from flexor motor neurons.

Results

Activity of the lump receptor during kicks

Few sensory spikes occurred spontaneously in the lateral nerve unless the tibia extended beyond 80° to activate the joint receptors, or moved into the fully flexed position. During a

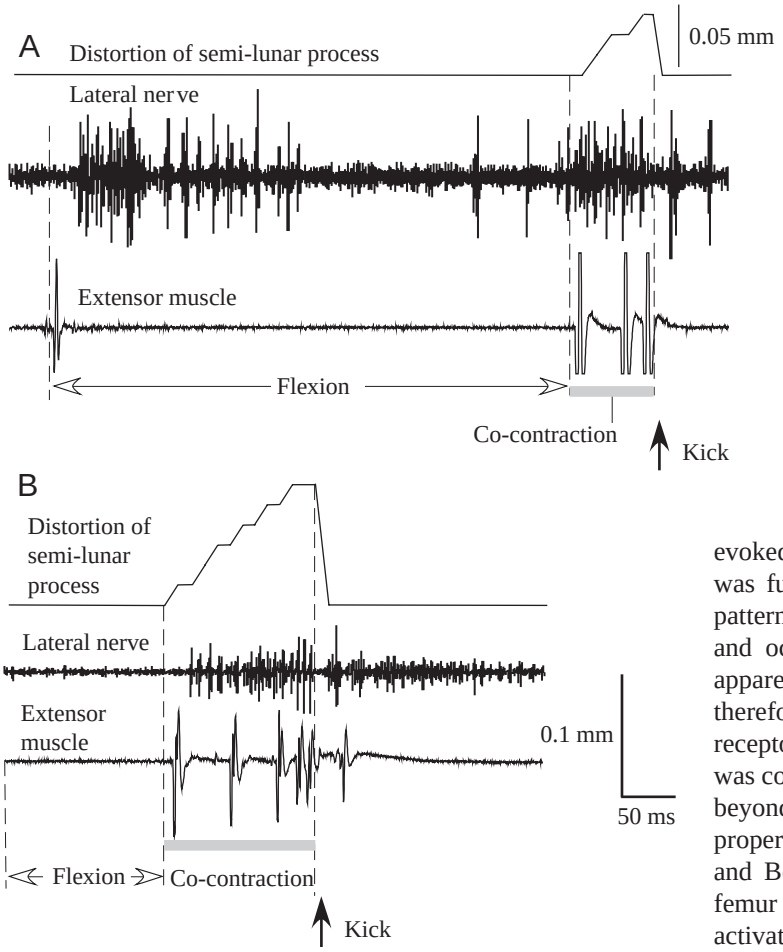

Fig. 2. Sensory signals and distortion of a semi-lunar process at the femoro–tibial joint in kicks with only short periods of co-contraction. (A) An initial spike in the fast extensor tibiae motor neuron (FETi) accompanied the movement of the tibia into a flexed position and was signalled by a burst of spikes in sensory neurons from the lump receptor. The tibia then remained flexed for 500 ms before a kick was generated by an 80 ms long co-contraction involving 3 FETi spikes. Only a small distortion of the semi-lunar process resulted and spikes from the lump receptor occurred at low frequency. (B) A kick following a 150 ms long co-contraction with 5 FETi spikes. The tibia was fully flexed about the femur before the displayed recording. The bending of the semi-lunar process was now twice as large and was accompanied by more sensory spikes during the co-contraction phase.

kick, however, spikes occurred in the lateral nerve during all three stages of the motor pattern (Fig. 1). When the tibia was actively drawn into its fully flexed position about the femur, sensory spikes occurred but there was no accompanying distortion of the femoro–tibial joint or bending of a semi-lunar process. Following the first FETi spike, which marked the onset of co-contraction by the extensor and flexor tibiae muscles, a semi-lunar process started to bend and sensory spikes in the lateral nerve occurred at a higher frequency. As the co-contraction phase proceeded, the frequency of FETi spikes increased and was accompanied by a progressive bending of the semi-lunar process and by a progressive increase in the frequency of sensory spikes in the lateral nerve. During the extension of the tibia in the kick, the spikes in the lateral nerve stopped briefly only to resume as the tibia flexed and extended at extended femoro–tibial angles as a rebound to the rapid movement (Burrows and Morris, 2001).

Interpretation of the sensory spikes

We believe the spikes in the lateral nerve that occurred during initial flexion and during the co-contraction phases of a kick were from the lump receptor. Spikes of similar amplitudes were activated in separate experiments (see Figs 4–7) by evoked contractions of the extensor and flexor muscles and occurred only when the tibia was fully flexed. They were also evoked by applying force to the flexor tendon when the tibia was fully flexed. In both of these phases of the kick motor pattern, two spike amplitudes could sometimes be recognised and occasionally larger amplitude spikes resulted from the apparent coincidence between these spikes. They could, therefore, correspond to the two sensory neurons of the lump receptor. By contrast, the spikes that occurred when the kick was completed could be evoked by forcibly extending the tibia beyond 80°, indicating that they had the same response properties as those of the joint receptors (Coillot, 1974; Coillot and Boistel, 1969). The sensory neurons from hairs on the femur innervated by the lateral nerve did not appear to be activated during kicking.

Correlation of lump receptor spikes with kicking movements

All kicks showed the same basic sequence of motor and sensory activity, though the detail varied between kicks of different strength and velocity of tibial movements (Figs 2, 3). In some kicks, the tibia was fully flexed about the femur for some time before the co-contraction phase was initiated. The initial flexion of the tibia was signalled by a burst of sensory spikes (Fig. 2A), but while the tibia was in the fully flexed position few spikes occurred in the lateral nerve (Fig. 2A,B). Kicks with a brief co-contraction phase that contained few FETi spikes led to the semi-lunar processes being bent by only a small amount (Fig. 2A). This was signalled by a low frequency of sensory spikes. If the co-contraction phase was longer and contained more FETi spikes then the bending of the semi-lunar processes was greater (Fig. 2B) and the frequency of spikes recorded in the lateral nerve was higher (Fig. 3A). In each kick, the frequency of spikes rose steadily during the co-contraction phase as the semi-lunar processes were progressively bent. The overall frequency of these sensory spikes reached 500 Hz in some kicks with a contribution from at least two neurons. Just before some kicks occurred, the frequency of the summed sensory spikes appeared to fall (Fig. 3A), because of the coincidence of spikes in the summed extracellular recording that was also reflected in their changing amplitude. When data for 10 kicks were pooled, there was a

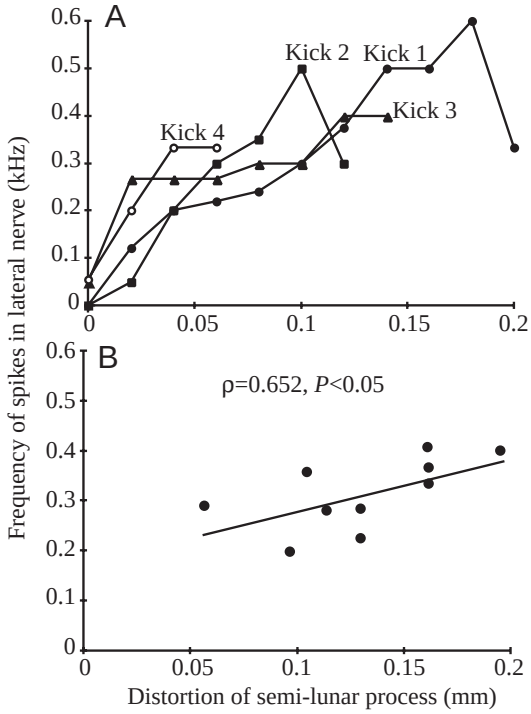

Fig. 3. Positive relationship between the frequency of spikes in the lateral nerve and the extent of distortion of a semi-lunar process during the co-contraction phase of kicking. (A) Plots of the correlation for 4 individual kicks. In kicks 1 and 2, the spike frequencies appear to decrease at the end of the co-contraction period due to the apparent synchronization of the sensory spikes in the extracellular recording at high frequencies. (B) Pooled data from 10 kicks by 3 locusts showing a positive relationship between the frequency of sensory spikes in the lateral nerve during co-contraction and the distortion of a semi-lunar process.

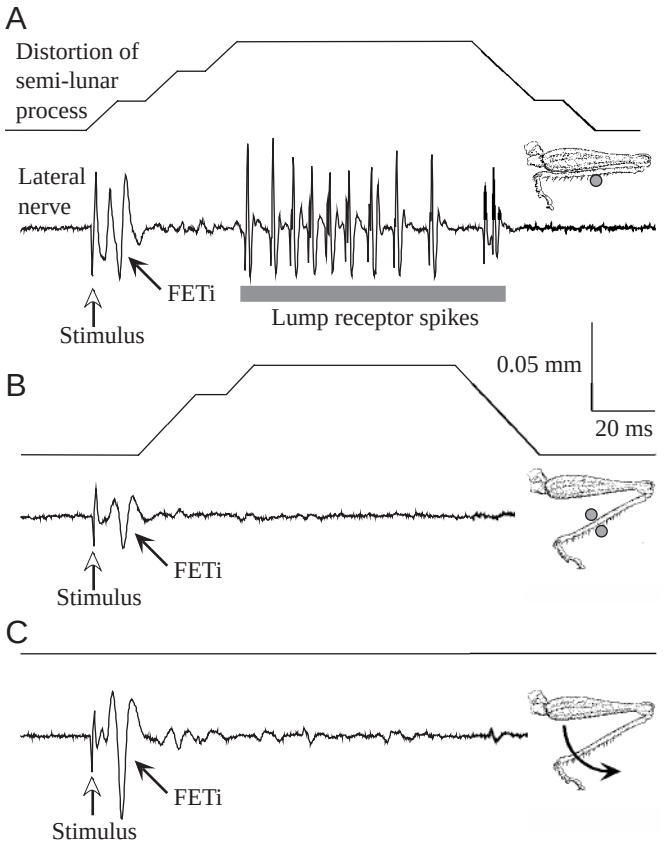

Fig. 4. Activity of the lump receptor during electrical stimulation of the extensor tibiae muscle with a single pulse. The spike of the fast extensor tibiae motor neuron (FETi) is visible as cross-talk in the recording from the lateral nerve; its waveform changes when the muscle contracts and moves. (A) The tibia was held in the fully flexed position and the resulting distortions of the femoro–tibial joint, measured from high speed images, were followed 40–50 ms after the stimulus by a burst of sensory spikes. (B) When the tibia was held in a partially extended position, the stimulus evoked a distortion of the semi-lunar process but no sensory spikes. (C) The tibia was free to move during the stimulus and there was no distortion and no sensory spikes.

positive correlation between the number (ρ=0.647, P<0.03, Spearman rank correlation test) and frequency (ρ=0.652, P<0.05, Fig. 3B) of spikes in the lateral nerve and the amount of bending in the semi-lunar processes. This suggests that during a kick the lump receptor monitors the bending of the semi-lunar processes, or some other correlated event(s) at the femoro–tibial joint.

Lump receptor responses to experimentally generated forces at the femoro–tibial joint

To determine what events at the femoro–tibial joint led to spikes in the lump receptor, we manipulated the different active forces and examined the effects of joint angle on these forces. Contractions were evoked in the femoral muscles. A single electrical stimulus to the extensor tibiae muscle activates the terminals of its two motor neurons (a slow, SETi and a fast, FETi) leading to an orthodromic spike in each that leads to a twitch contraction of the muscle fibres. The electrical stimulus also evokes antidromic spikes that are carried toward the metathoracic ganglion. The antidromic spike in FETi activates a monosynaptic pathway to the flexor motor neurons that can evoke flexor spikes and a contraction of the flexor muscle (Burrows et al., 1989; Hoyle and Burrows, 1973).

The co-contraction phase was simulated in 7 locusts by electrically stimulating the extensor tibiae muscle with the tibia held fully flexed against the femur and unable to extend (Fig. 4A). Each stimulus and the resulting spike in FETi was followed by a transient bending of the semi-lunar processes, distortion of the dorsal, distal cuticle of the femur and, some 40–50 ms later, by a burst of spikes in the lump receptor. The peak frequency of the spikes was about 200 Hz. The spikes continued throughout the 80 ms period that the semi-lunar processes were maximally bent, and stopped when the bending relaxed. If, however, the same stimulus was applied to the extensor muscle when the tibia was clamped at an angle greater than 20° and thus unable to extend further, the semi-lunar processes were still bent but no sensory spikes occurred in the

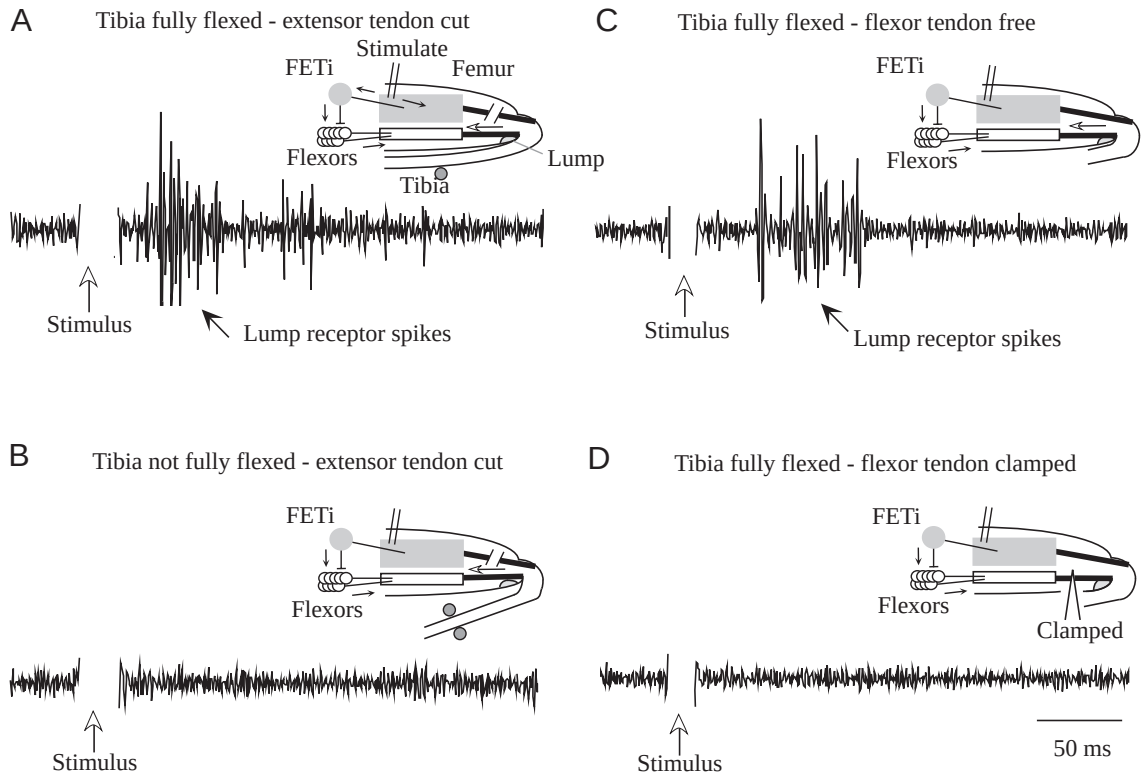

Fig. 5. Effect of manipulating the actions of the extensor and flexor muscles on the signalling by the lump receptor. (A,B) The extensor tendon was cut in the distal femur so that stimulation of the extensor muscle exerted no direct force on the joint. Instead the stimulus activated flexor tibiae motor neurons through the central, monosynaptic connections that the fast extensor tibiae motor neuron (FETi) makes with them, and caused a contraction of the flexor muscle. (A) With the tibia held in the fully flexed position and the flexor tendon free to move, a burst of spikes from the lump receptor followed the stimulus. (B) The tibia in the same locust was held in a partially extended position, and the stimulus now did not lead to sensory spikes. (C,D) A second locust in which the extensor tendon was intact. (C) The flexor tendon exerted force on the joint fixed in the fully flexed position. The stimulus was accompanied by a burst of sensory spikes. (D) The flexor tendon was clamped so that force could not be transmitted through it to the joint. No sensory spikes followed the stimulus. The inset diagrams show the experimental arrangement. The solid arrows show the flow of effects from the evoked FETi spike; the open arrows, the movement of the flexor tendon. The recording during the stimulus has been truncated.

lateral nerve (Fig. 4B). Finally, if the same stimulus was given to the extensor muscle when the tibia was allowed to extend freely, then the semi-lunar processes did not bend and no sensory spikes occurred (Fig. 4C). These experiments indicate that bending of the semi-lunar processes or cuticular distortions at the femoro–tibial joint are not directly responsible for evoking the sensory spikes.

We, therefore, tested whether forces generated in the tendons of either the flexor or extensor tibiae muscles were responsible (Fig. 5). First, in three locusts, the extensor tendon was cut in the distal tibia and a single electrical stimulus was delivered to the muscle as above. When the tibia was held fully flexed about the femur, an FETi spike was followed by a burst of spikes from the lump receptor similar to those seen in an intact leg (Fig. 5A). Force generated by contraction of the extensor muscle could not be transmitted to the femoro–tibial joint but the flexor muscle was activated through the central pathway. Repeating the same stimulus with the tibia extended by more than 20° and free to move did not evoke any sensory spikes (Fig. 5B). In a further three locusts, the flexor tendon was exposed in the distal femur so that it could be clamped reversibly, but the extensor tendon was intact. When the tibia was held fully flexed about the femur and the flexor tendon was unclamped, the usual burst of spikes from the lump receptor followed stimulation of the extensor muscle (Fig. 5C). When the flexor tendon was clamped so that force or movement generated in the flexor muscle could not be transmitted to the joint, no sensory spikes were evoked (Fig. 5D). These experiments indicate that the lump receptor responds to force or movements of the flexor tendon but not to the force generated by the extensor muscle.

This conclusion was tested further in five locusts by pulling on the flexor tendon to apply different forces in the direction that would cause the tibia to flex (Fig. 6). With the tibia in the fully flexed position, pulling on the tendon evoked a burst of spikes in the sensory neurons from the lump receptor (Fig. 6A). If the tibia was fixed at an angle of 20° or more, however, the same amount of applied force did not evoke any sensory spikes (Fig. 6B). This observation therefore confirms the result of Heitler and Burrows (1977b).

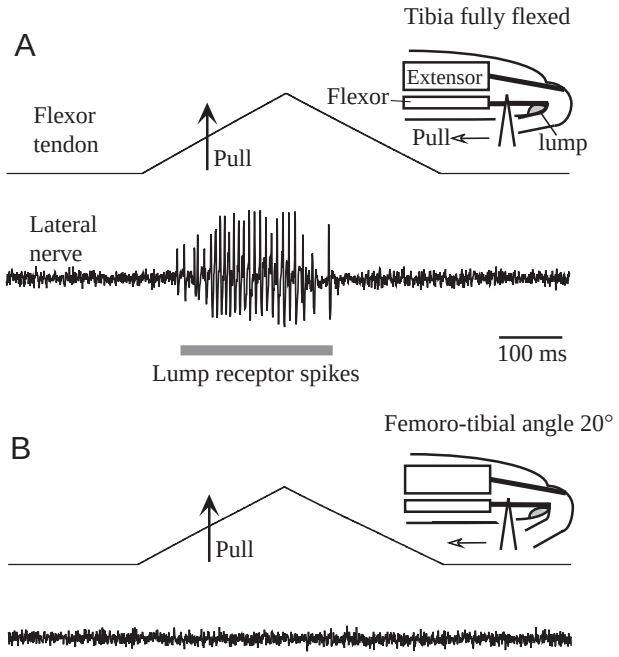

Fig. 6. Activity of sensory neurons from the lump receptor recorded in the lateral nerve in response to forces applied to the tendon of the flexor muscle. (A) With the tibia fully flexed, pulling on the flexor tendon evoked a burst of sensory spikes. (B) With the tibia extended by 20°, no spikes followed the applied pull.

Effect of flexor tibiae contraction on lump receptor activity

The muscle fibres that form the main body of the flexor muscle are grouped into 10–11 pairs of bundles that insert onto a common central tendon (Sasaki and Burrows, 1998). These muscle bundles are innervated by different sets of motor neurons, the axons of which run in small side branches of N5B2. Cutting particular nerve branches can, therefore, selectively abolish the contraction of certain bundles and could be used to test whether all parts of the muscle contributed to the excitation of the lump receptor. A nerve branch was cut in the middle of the femur of three locusts, dividing the muscle into an innervated proximal part and a denervated distal part (Fig. 7A,B). Before the nerve was cut, an electrical stimulus to the extensor tibiae muscle with the tibia fixed in the fully flexed position, evoked a burst of spikes in the sensory neurons from the lump receptor (Fig. 7A). Following the cut, when only the proximal part of the muscle could contract, the same stimulus evoked a burst of spikes of lower frequency (Fig. 7B).

To test whether selective contraction of distal flexor muscle bundles was equally effective, the flexor tendon was cut between the 4th and 5th pairs of muscle bundles with N5B2 intact (Fig. 7C,D). In the control experiment before the cut was made, stimulation of the extensor muscle evoked a burst of spikes in the lump receptor (Fig. 7C). After the cut, however, the force generated by the distal muscle bundles alone did not evoke spikes in the lump receptor (Fig. 7D).

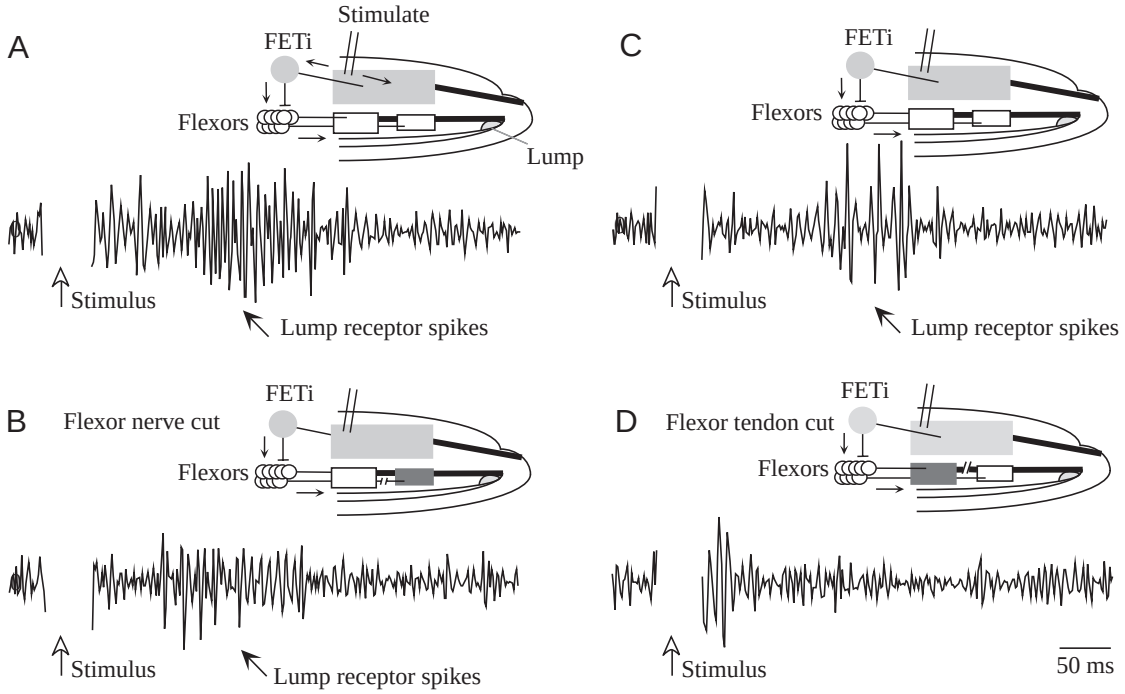

Fig. 7. Different effects of contractions by proximal and distal bundles of the flexor muscle on signalling by the lump receptor. (A) A single stimulus to the extensor muscle in an intact leg with the tibia fully flexed was followed by a burst of spikes from the lump receptor. (B) The same locust but with the flexor nerve (N5B2) cut in the middle of the femur, thereby preventing the distal muscle bundles from contracting. The contraction of proximal muscle bundles caused a burst of sensory spikes. (C,D) A second locust. (C) In the intact leg, sensory spikes followed the stimulus with the tibia fully flexed. (D) The flexor tendon was cut between the proximal and distal bundles of fibres so that the force developed by proximal fibres was not transmitted to the joint. Contraction of the distal muscle bundles was not followed by spikes from the lump receptor.

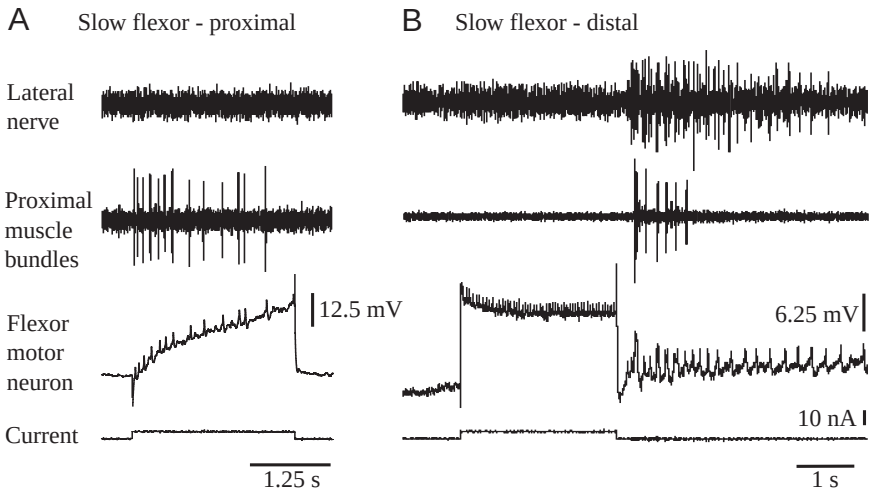

Fig. 8. Effects of the force produced by the action of individual flexor motor neurons on the activity of sensory neurons from the lump receptor. A pulse of depolarising current was injected into the cell body of a motor neuron to evoke spikes. (A) Spikes in a slow flexor motor neuron innervating the proximal muscle bundles did not elicit spikes in the lump receptor. (B) Similarly a high frequency of spikes in another slow motor neuron innervating distal muscle bundles did not evoke sensory spikes. A later burst of spikes in fast flexor motor neurons, not directly related to the applied depolarisation, caused muscle activity and sensory spikes. The bridge is unbalanced in the intracellular recordings.

Individual motor neurons were then activated by intracellular injection of current into their somata to test whether the contraction they evoked activated the lump receptor. Intracellular stimulation of a slow flexor motor neuron evoked spikes that were also recorded in proximal muscle bundles, but did not activate the lump receptor (Fig. 8A). A high frequency of spikes in a slow flexor motor neuron innervating the distal muscle bundles also did not evoke sensory spikes in the lump receptor (Fig. 8B). By contrast, when several fast-like flexor motor neurons innervating the proximal muscle fibres spiked spontaneously after the applied depolarisation, the contractions they evoked activated spikes in sensory neurons from the lump receptor (Fig. 8B).

Effect of lump receptor on flexor tibiae motor neurons

To determine whether sensory feedback from the lump receptor could regulate the action of flexor motor neurons, intracellular recordings were made from members of the pool of nine flexor motor neurons while the lump receptor spikes were evoked by pulling on the flexor tendon (Fig. 9). Three of seven fast-like flexor motor neurons were hyperpolarized when the lump receptor spiked (Fig. 9A). By contrast, the remaining four fast-like motor neurons showed little or no change in membrane potential during sustained spiking by the lump receptor (Fig. 9B). Similarly, in four slow motor neurons, no change in their synaptic inputs, or in the frequency of a tonic sequence of their spikes (Fig. 9C), could be detected during spikes of the lump receptor evoked by force applied to the flexor tendon.

The spikes from the lump receptor, that resulted in a hyperpolarization in three of seven fast flexors, also altered the synaptic inputs in these motor neurons generated by spikes in FETi (Fig. 10A,B). In each of the three flexor motor neurons, the excitatory synaptic potentials (EPSPs) evoked by FETi spikes were reduced in amplitude when the flexor tendon was pulled and spikes occurred in the lump receptor. The reduction was seen in individual EPSPs compared with the same position in the sequence (Fig. 10B) or in averages of the responses from before, during and after the applied stimulus. The lump receptor could, therefore, reduce the probability of spikes being generated in flexor motor neurons and, thus, act in a negative feedback loop to reduce flexor tension during the co-contraction phase of a kick.

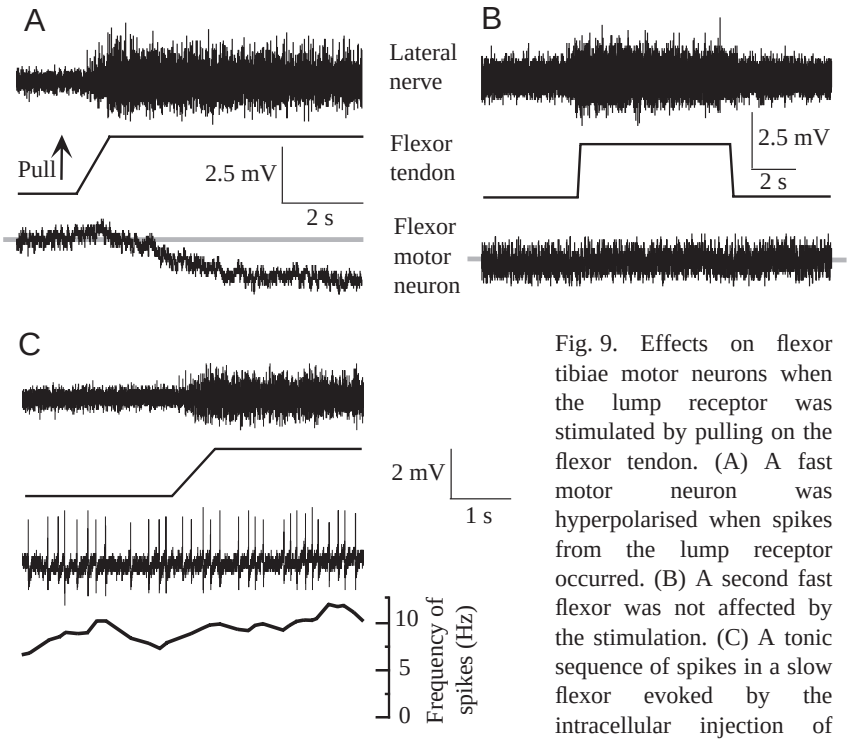

Fig. 9. Effects on flexor tibiae motor neurons when the lump receptor was stimulated by pulling on the flexor tendon. (A) A fast motor neuron was hyperpolarised when spikes from the lump receptor occurred. (B) A second fast flexor was not affected by the stimulation. (C) A tonic sequence of spikes in a slow flexor evoked by the intracellular injection of depolarising current was not altered by spikes from the lump receptor. Grey lines indicate the membrane potential of a motor neuron before stimulation of the lump receptor.

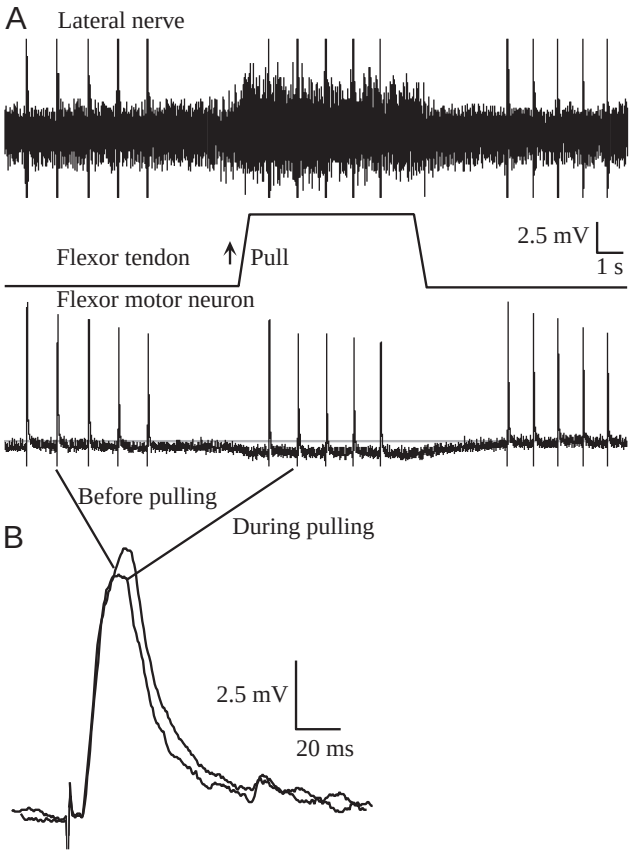

Fig. 10. Effects of spikes from the lump receptor on the synaptic connection between the fast extensor tibiae motor neuron (FETi) and flexor motor neurons. (A) The experimental protocol. Antidromic spikes were evoked in FETi by stimulation of the extensor muscle. 5 stimuli at intervals of 1 s were given before the flexor tendon was pulled. 4 s after the last stimulus the tendon was pulled and 5 more stimuli were delivered. The motor neuron was hyperpolarized by the sensory spikes and the EPSPs were reduced in amplitude. Repetition of the electrical stimuli after the movement of the tendon showed that the EPSPs recovered to their previous amplitude. (B) Comparison of the second EPSP before the sensory stimulus with the second EPSP during the sensory spikes (grey trace) shows the changes in amplitude.

Discussion

The sensory neurons from the lump receptor in a locust hind leg are excited during the co-contraction phase of a kick and their pattern of spikes correlates directly with the distortions of the cuticle. The muscular force generated by spikes in individual flexor tibiae motor neurons can elicit spikes in the lump receptor, which in turn regulate the output of specific members of the flexor motor pool in a polysynaptic, negative feedback loop. In this way the signalling by the lump receptor may limit the force generated in a kick.

Signalling by the lump receptor

The sensory neurons of the lump receptor of a hind leg are only excited when the femoro–tibial joint is fully flexed. When the joint is in this position, movements of the flexor tendon or force exerted on it by contractions of the flexor muscle, or co-contractions with the extensor muscle excite the lump receptor. When the tibia is not fully flexed, co-contractions of the extensor and flexor muscles distort the femoral cuticle of the joint but do not excite the lump receptor. Similarly, contractions of the extensor alone do not excite the lump receptor although they can cause cuticular distortions. The most likely stimulus is the movement of the flexor tendon past the receptor, or its downward pressure on it when the tibia is moving toward or is in the fully flexed position. It is only at full flexion of the tibia that the flexor tendon engages fully with the cuticular lump in which the receptor lies (Heitler and Burrows, 1977b). The lump receptor, therefore, signals the extent of a co-contraction in a kick by directly monitoring the flexor tendon and only indirectly the distortions of the femoro–tibial joint caused by the muscle contractions. The restriction of the lump receptor to signalling at full tibial flexion suggests that it functions only during jumping and kicking. The energy required for these movements cannot be generated unless the tibia is fully flexed and the flexor tendon is locked over the cuticular lump (Heitler, 1977). By contrast, the normal walking movements do not involve full flexion of a hind leg and signalling by the lump receptor would not be expected.

Multipolar receptors also occur at the femoro–tibial joint of the middle legs of a locust (Mucke, 1991; Williamson and Burns, 1978) even though these legs do not have the same structural specialisations for jumping and kicking as do the hind legs. Most notably they lack a femoral lump, so the lines of action of the muscle tendons are different (Heitler, 1974). Three of these receptors, as in the hind leg, respond to extension of the tibia beyond 80° but the action of the other two neurons, which attach to the ventral arthrodial membrane of the femur, has not been reported (Williamson and Burns, 1978). Our preliminary observations indicate that these two receptors respond to direct pressure on the flexor tendon or to movements of the arthrodial membrane when the flexor tendon of a middle leg moves between angles of 20–30°. In these legs, therefore, these ventral receptors may signal force or movements of the flexor tendon during normal walking, when clinging to a twig or hanging on a grass stem. The specialisations of the hind legs for kicking and jumping would then be seen to have been accompanied by a changed and more restricted role for their equivalent receptors in monitoring events during co-contractions leading to kicking and jumping.

Negative sensory feedback loops with a pool of flexor motor neurons

The flexor tibiae muscle consists of 10–11 pairs of muscle bundles that insert onto a common central tendon. Different muscle bundles are innervated by different numbers of excitatory motor neurons (Sasaki and Burrows, 1998) from the pool of approximately nine flexor motor neurons (Phillips, 1980). The proximal muscle bundles are innervated by seven motor neurons, including two fast and three intermediate motor neurons. The muscular force generated by spikes in one of

these fast motor neurons can excite the lump receptor. The contractions of these muscle bundles are then regulated by a negative feedback loop acting through a polysynaptic pathway to inhibit particular fast flexor motor neurons. In this way, the activity of certain flexor motor neurons innervating particular parts of the muscle can excite the lump receptor while simultaneously being regulated by sensory feedback from it. The feedback loops formed by the lump receptor act in parallel to those from other receptors, such as the femoral chordotonal organ at the femoro–tibial joint (Field and Burrows, 1982; Matheson and Field, 1990; Usherwood et al., 1968), or the tension receptor in the most distal bundle of the flexor tibiae muscle (Matheson and Field, 1995).

Explanations for the large number of neurons in a particular motor pool are thought to lie in a subdivision of function among the members, a subdivision of action by different parts of the muscle by virtue of different innervation patterns, intrinsic differences in the properties of the muscle fibres, or a combination of these factors (Skorupski et al., 1992). Subdivision of connections and hence possible actions are seen within the flexor tibiae motor pool. For example, campaniform sensilla on the tibia of a middle leg directly excite a fast but not a slow flexor motor neuron (Newland and Emptage, 1996). Similarly, fast flexor motor neurons are excited only by fast imposed movements of the apodeme of the femoral chordotonal organ in a hind leg, whereas the opposite is true for a slow motor neuron (Burrows, 1987; Field and Burrows, 1982). Although the different flexor motor neurons receive many synaptic inputs in common, each has specific dynamic responses to movements of the femoro–tibial joint (Newland and Kondoh, 1997). The specific feedback loops made by the lump receptor suggest further subdivision of function among members of the flexor motor neuron pool. Fast-like flexor motor neurons innervating proximal muscle fibres excite the lump receptor and are themselves inhibited by the sensory signals from it. Slow flexor motor neurons are less likely to excite the lump receptor and are apparently unaffected by feedback from it.

How might sensory signals from the lump receptor contribute to kicking?

Cutting the lateral nerve containing the axons of the joint receptors reduces the probability of evoking a kick by some 30%, but removing the femoral chordotonal organ causes a 70% reduction (Jellema et al., 1997). Section of the lateral nerve, however, has no effect on the duration of the co-contraction phase or the number of spikes produced by FETi during the co-contraction (Jellema and Heitler, 1997) but does result in more spikes in FETi after the kick and a prolongation of the time before the tibia is flexed once again. The latter effects have, however, been attributed to the three extension sensitive joint receptors and not to the lump receptor (Jellema and Heitler, 1997). Could the high frequency of spikes in the sensory neurons from the lump receptor towards the end of the co-contraction contribute to ending this phase of the motor pattern and allow a kick to occur? At the end of the co-contraction, excitatory flexor motor neurons are rapidly hyperpolarized and stop spiking and at the same time, the two inhibitory motor neurons that innervate the flexor muscle are excited (Burrows, 1995; Heitler and Burrows, 1977a). This allows the force developed by the extensor muscle during the co-contraction to be delivered rapidly and propel the extension of the tibia. The timing of the sensory signals from the lump receptor suggests that they could progressively reduce the effectiveness of excitation to the flexors during co-contraction and to the final inhibition. The distribution of the polysynaptic inhibitory pathway to particular fast-like flexor motor neurons suggests that other parallel pathways also operate. It will, therefore, be important to determine whether the lump receptor makes connections with the many interneurons that are involved in control of leg movements. The negative feedback loops could limit the force that is generated in a kick to that which the cuticle can sustain, and thus avoid damage to the joint, particularly in recently moulted locusts in which hardening is not yet complete.

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