The Journal of Experimental Biology **199**, 613–625 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JEB0105

PERIPHERAL CONTROL OF THE GAIN OF A CENTRAL SYNAPTIC CONNECTION BETWEEN ANTAGONISTIC MOTOR NEURONES IN THE LOCUST

T. JELLEMA AND W. J. HEITLER
The Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland

Accepted 24 October 1995

Summary

The metathoracic fast extensor tibiae (FETi) motor neurone of locusts is unusual amongst insect motor neurones because it makes output connections within the central nervous system as well as in the periphery. It makes excitatory chemical synaptic connections to most if not all of the antagonist flexor tibiae motor neurones. The gain of the FETi–flexor connection is dependent on the peripheral conditions at the time of the FETi spike. This dependency has two aspects. First, sensory input resulting from the extensor muscle contraction can sum with the central excitatory postsynaptic potential (EPSP) to augment its falling phase if the tibia is restrained in the flexed position (initiating a tension-dependent reflex) or is free to extend (initiating a movement-dependent resistance reflex). This effect is thus due to simple postsynaptic summation of the central EPSP with peripheral sensory input. Second, the static tibial position at the time of the FETi spike can change the amplitude of the central EPSP, in the absence of any extensor muscle contraction. The EPSP can be up to 30 % greater in amplitude if FETi spikes with the tibia held flexed rather than extended. The primary sense organ mediating this effect is the femoral chordotonal organ. Evidence is presented suggesting that the mechanism underlying this change in gain may be specifically localised to the FETi–flexor connection, rather than being due to general position-dependent sensory feedback summing with the EPSP. The change in the amplitude of the central EPSP is probably not caused by general postsynaptic summation with tonic sensory input, since a diminution in the amplitude of the central EPSP caused by tibial extension is often accompanied by overall tonic excitation of the flexor motor neurone. Small but significant changes in the peak amplitude of the FETi spike have a positive correlation with changes in the EPSP amplitude, suggesting a likely presynaptic component to the mechanism of gain control. The change in amplitude of the EPSP can alter its effectiveness in producing flexor motor output and, thus, has functional significance. The change serves to augment the effectiveness of the FETi–flexor connection when the tibia is fully flexed, and thus to increase its adaptive advantage during the co-contraction preceding a jump or kick, and to reduce the effectiveness of the connection when the tibia is partially or fully extended, and thus to reduce its potentially maladaptive consequences during voluntary extension movements such as thrusting.

Key words: grasshopper, femoral chordotonal organ, *Schistocerca gregaria*, jumping, kicking.

Introduction

The definitive function of motor neurones is to make output in the periphery to muscles, but in some systems motor neurones may also make output within the central nervous system onto other motor neurones or interneurones. A well-known example is the motor neurones in the spinal cord of vertebrates, which interact through inhibitory Renshaw cells (Renshaw, 1940). Within the arthropod groups, direct electrical and chemical synapses between motor neurones are abundant in crustaceans (Mulloney and Selverston, 1974; Heitler, 1978; Skorupski and Sillar, 1988) but extremely rare in insects. The only insect motor neurone known to have a central chemical synaptic output is the fast extensor tibiae motor neurone (FETi) located in the metathoracic ganglion of the locust. FETi innervates the extensor tibiae muscle of the hindleg, but it also makes central excitatory connections with most, if not all, of the antagonistic flexor motor neurones, which innervate the flexor tibiae muscle of the same leg (Hoyle and Burrows, 1973; Heitler and Burrows, 1977*b*). The primary central connection involves only a single synaptic delay, though interneurones may be involved in subsidiary pathways (Burrows *et al.* 1989).

The amplitude and waveform of the central EPSP can be altered by varying the position of the tibia about the femur (Heitler and Burrows, 1977*b*). Central EPSPs evoked while the leg is held in a flexed position are of larger amplitude and longer duration than those evoked during an imposed extension. Differences in sensory feedback mediated by receptors in the hindleg must be responsible for these changes in the gain of the connection. The chordotonal organ in the distal femur (FCO),

which monitors leg movement and position (Usherwood *et al.* 1968), and the campaniform sensilla on the proximal tibiae, which monitor cuticle strain (Burrows and Pflüger, 1988) are, amongst others, candidate sources for the modulatory influences. The central site(s) and mechanism(s) of the changes in gain are unknown. A positive correlation between FETi spike amplitude and the amplitude of the EPSP has been shown (Heitler and Burrows, 1977*b*; Burrows *et al.* 1989), and this finding is compatible with a presynaptic mechanism, but it is by no means conclusive evidence for it.

The purpose of the present report is to characterise more fully this peripheral control of the central synapse. Two primary peripheral conditions affecting the shape of the central EPSP may be discriminated. First, the tibial position at the moment of FETi activation may mediate continuous tonic influences which directly affect the FETi–flexor connection itself. Second, the peripheral muscle contraction caused by the FETi spike may cause a variety of sensory inputs which sum with the central EPSP. In the latter case, different sensory feedback will be elicited depending on whether the leg is free to move, causing movement-related reflexes, or whether it is held in a fixed position, causing tension-related reflexes which vary with the position in which the leg is held. In the intact preparation, these individual components all sum with the central EPSP, and it is very difficult to identify them separately. To overcome this problem, two main approaches have been taken: (1) laser photo-axotomy of FETi, and (2) manipulation of the tendon of the FCO.

Laser photo-axotomy (Miller and Selverston, 1979) of FETi allows the axon of FETi to be cut without affecting any of the other structures (Heitler, 1995). This has two experimental advantages. First, FETi spikes can activate the central EPSP without eliciting any muscle tension, so position-dependent peripheral effects can be separated from tension- and movement-dependent effects. Second, and conversely, the tension- and movement-dependent effects can be elicited by muscle stimulation without an antidromic spike in FETi and thus without any central EPSP.

The FCO is one of the main sensory systems signalling tibial position and movement, and its afferents form direct synaptic connections with FETi, flexor motor neurones and interneurones (Burrows, 1987, 1988) and indirect synaptic connections with each other (Burrows and Matheson, 1994). After disconnection from the tibia, the FCO tendon (apodeme) can be manipulated experimentally to study the effects of the FCO in isolation from all other incoming sensory signals (cf. Field and Burrows, 1982).

Materials and methods

Animal and preparation

Adult locusts (*Schistocerca gregaria* Forskål) of either sex were taken from a crowded colony. The locust was restrained on its back in Plasticene. The femur of the right hindleg was rotated so that its anterior surface was uppermost, and was firmly embedded while leaving the tibia free to move. The ventral thoracic cuticle was dissected to expose the meso- and metathoracic ganglia. All peripheral nerves from these ganglia were cut except for nerve 5 innervating the ipsilateral hindleg. This reduces variability introduced by sensory input from other legs and also prevents contractions of the thoracic muscles. The metathoracic ganglion was stabilised on a wax-coated stainless-steel platform, and its sheath was treated with a few crystals of protease (Sigma type XIV). The thoracic cavity was subsequently perfused with a constant flow of locust saline (Hoyle and Burrows, 1973) at 20–22 °C.

FCO manipulation

The distal part of the tendon of the FCO, including its attachment to the tibia, was exposed by cutting a window on the anterior face of the femoral–tibial joint just opposite the heavily sclerotized semilunar process, while the leg was held in the extended position. With the leg still extended, the tendon was grasped between the tips of forceps attached to an electromagnetic vibrator (Ling, type 101) controlled by a function generator, before cutting it distally. In this way, an open loop was created driven by the function generator so that the tendon could alternately be stretched and relaxed while the leg was kept in a fixed position. Stretching the tendon corresponds to a movement which normally happens during flexion of the femoral–tibial joint, while relaxing it corresponds to a movement normally happening during extension of the joint. For most of the range between full flexion and full extension of the leg, a 500 μm movement of the tendon corresponds to a 40° movement of the femoral–tibial joint (Field and Burrows, 1982). A 2.2 mm movement of the tendon was used to switch between the two extreme positions. Only ramp-and-hold movements were applied. The FCO flexor strand was left intact. Because the leg was kept in a fixed position during the manipulations of the tendon, the flexor strand is unlikely to contribute to any differences found due to the tendon manipulations.

Recording and stimulation

Intracellular recordings from the somata of FETi and the flexor motor neurones were made using thin-walled microelectrodes, filled with 2 mol l^{-1} potassium acetate (d.c. resistance 20–40 MΩ). Intracellular recordings in the neuropile of these neurones were made from the dorsal side of the metathoracic ganglion, for which purpose the ganglia were turned over on the platform. There is sufficient slack in the connectives and nerve 5 to achieve this without cutting these nerves. The route from the dorsal surface to the neuropile region is shorter than that from the ventral surface, and the dorsal sheath is thinner. Successful recording was further facilitated by using thick-walled microelectrodes (resistance 80–100 MΩ). The neuropile recording sites were located halfway between the FETi soma and the entrance of nerve 5 into the ganglion, at a depth of 200–250 μm. Flexor neuropile sites were located 50–100 μm medial to FETi neuropile sites (cf. Burrows *et al.* 1989). Flexor and extensor EMGs were recorded with pairs of 50 μm copper wires, insulated except at

their tips. Antidromic spikes were evoked in FETi by stimulating its peripheral axonal branches *via* the EMG wires placed in the extensor muscle. Orthodromic spikes were evoked by injecting depolarising current into FETi *via* the recording microelectrode. Motor neurones were identified by (1) injecting depolarising current into them and observing any movements of the leg or activity in the EMG, (2) stimulating the appropriate muscle to produce an antidromic spike in the soma, (3) correlating spontaneous spikes with EMG activity. The results are based on recordings in the FETi and a flexor motor neurone (FlTi) in 60 locusts. Flexor motor neurones were not categorised by type (fast, slow etc.).

Laser photo-axotomy of FETi

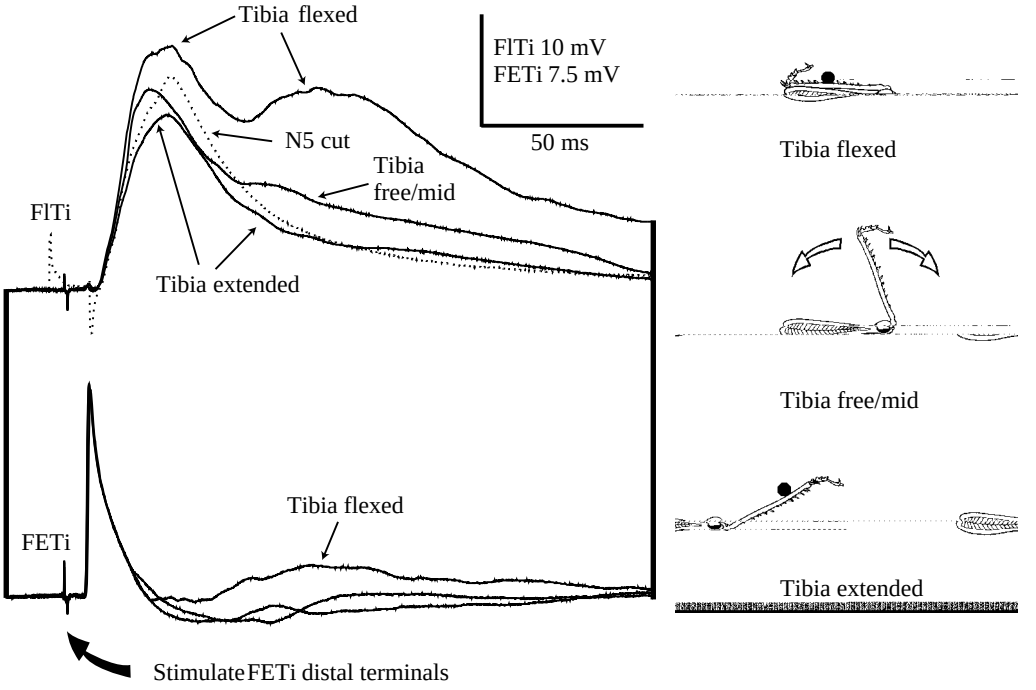
To separate the effects of sensory feedback from the central EPSP, dye-mediated laser photo-axotomy of FETi was applied (Miller and Selverston, 1979; Heitler, 1995). This totally de-efferents the extensor muscle, but has no effect on adjacent axons running in the same root, and leaves unchanged both the central FETi properties and the sensory/mechanical properties of the leg. When the FETi axon terminals in the extensor muscle are stimulated, the individual peripheral components caused by leg movement (condition 1: leg free to move) and by tension (condition 2: leg in fixed position) can be recorded in a flexor motor neurone, in the absence of the central component. The effect of the leg position alone (condition 3) can be studied by evoking orthodromic spikes in the axotomised FETi while manipulating the leg position. Both the central synaptic connection and the sensory feedback induced by leg position are activated, while tension and/or movement feedback are absent.

Data analysis

Data were digitally stored on tape (Biologic DTR-1802) for later analysis. Averages were made from 8–12 consecutive responses (0.5 Hz). At least six stimuli were allowed to occur from the start of stimulation or from a change in the FCO tendon position before averaging started, in order to allow the amplitude of the EPSP to stabilise. Records were displayed and averages were made using an EGAA data-acquisition system (RC Electronics).

Results

A spike in FETi evokes a depolarisation in flexor tibiae motor neurones by means of a monosynaptic excitatory central connection (Hoyle and Burrows, 1973; Heitler and Burrows, 1977*b*; Burrows *et al.* 1989). The shape of this depolarisation is strongly influenced by the peripheral conditions at the moment of the FETi spike (Fig. 1). In many cases there are two clear components to the depolarisation: an initial component with a latency from the FETi spike to the peak flexor depolarisation of about 22 ms and a later component with a latency of 70–100 ms. The amplitude of the initial component depends on the degree of tibial flexion at the time of the FETi spike; when the tibia is flexed, the amplitude is greater than



when it is extended. In eight preparations, the average peak amplitude of the initial EPSP when the tibia was held fully flexed was 15.6±4.2 mV (mean ± S.D.), whereas when the tibia was held fully extended the peak amplitude was 10.1±3.1 mV, i.e. a decrease of 35 %. When the leg was in the mid position, the EPSP amplitude was intermediate. The amplitude of the second component also depends upon the peripheral conditions; if the tibia is held flexed, there is a substantial depolarisation, whereas if the tibia is held extended, the second component is almost completely abolished. If the tibia starts in the mid position, but is free to move, the second component is of intermediate amplitude. If nerve 5 is cut, thus abolishing all motor output to and sensory feedback from the leg, and FETi is induced to spike by injecting depolarising current, the central EPSP has only the single initial component and this is of intermediate amplitude, similar to that occurring when the tibia is in the mid position (Fig. 1).

These data suggest that the initial component of the flexor response is due to the central FETi-induced EPSP itself, whereas the second component is due to peripheral input initiated in response to the extensor tibiae muscle contraction. If this is so, then the variation in the amplitude of the second component is due to the different types of sensory feedback depending on whether the tibia is free to move or is fixed, and if the latter, the position in which it is fixed. The variation in the amplitude of the first component may be due to tonic sensory input signalling tibial position which occurs independently of the FETi spike. These hypotheses will now be explored further.

Gain changes induced by extensor muscle contraction

Sensory input impinging on flexor motor neurones in response to extensor muscle contraction (the putative second component of the compound response) can be separated from the central EPSP induced by the FETi spike (the putative initial component) by photo-ablation of the FETi axon. This cuts the FETi axon without damaging either the central or peripheral regions of the FETi axon, or any of the other motor or sensory axons which run in the same nerve root (Heitler, 1995). The peripheral portion of the FETi axon can then be stimulated, thus inducing extensor muscle tension, without eliciting an antidromic spike in FETi and hence also without inducing the central flexor EPSP. This experiment was performed with the tibia in three conditions: free to move from the mid position, held fully flexed and held fully extended. The FETi and flexor motor neurone responses were compared before and after FETi axotomy (Fig. 2).

The tibia is free to move (Fig. 2A)

When the FETi axon is intact, stimulating the FETi axon terminals in the extensor muscle with the tibia free to move initiates an antidromic spike in FETi and a contraction in the muscle, causing tibial extension. The antidromic spike activates the central synapse, while tibial extension activates movement detectors in the joint which mediate a movement-dependent input. Following FETi photo-axotomy, the antidromic spike cannot reach the ganglion; thus, no central EPSP is evoked. Now only the peripheral movement-dependent input is recorded in the motor neurones, consisting of a depolarisation in the flexor motor neurone and a hyperpolarisation in FETi. This constitutes the synaptic basis for a classic form of resistance reflex. The flexor EPSP mediated by the movement detectors after axotomy starts about 16 ms later than the central EPSP observed prior to axotomy and is of smaller amplitude. The inhibitory sensory input to FETi is delayed relative to the excitatory sensory input to the flexor by about 2 ms. The movement-dependent inputs are likely to be mediated by the FCO (Field and Burrows, 1982) and the joint receptors with axons in the lateral nerve (Coillot and Boistel, 1968; Heitler and Burrows, 1977*b*). (The lateral nerve is a branch of nerve 5, and hence is intact in these experiments.)

The tibia is held in a fixed flexed position (Fig. 2B)

Stimulating FETi with the tibia held in the fully flexed position initiates an extensor muscle contraction and tension-dependent cuticle strain in the joint region, but no extension movement of the tibia. Prior to photo-axotomy, there is an antidromic spike in FETi followed by a small depolarisation. In the flexor motor neurone, there is a substantial depolarisation caused by the central EPSP, which sums with a prolonged excitatory sensory input. The contribution of the sensory input alone is revealed following photo-axotomy. There is still a substantial excitatory response in the flexor, but this is delayed relative to the start of the central EPSP (occurring before axotomy) by about 19 ms and does not depolarise the flexor to the same level. In this preparation, there is little or no excitatory response in FETi. The tension-dependent sensory input may be mediated in part by campaniform sensilla responding to cuticle strain (Burrows and Pflüger, 1988), but may also include a component from the FCO. The extensor muscle contraction causes a slight proximal movement of the femoral–tibial joint pivot (Bennet-Clark, 1975) and, since the tendon of the FCO attaches to the tibial head, this could induce a ‘pseudo’ resistance reflex as proximal movement of the tendon mimics tibial extension.

The tibia is held in a fixed extended position (Fig. 2C)

Stimulating FETi with the tibia held in the fully extended position initiates an extensor muscle twitch, but there is little tension developed because the muscle is not loaded, and there is no tibial movement. Prior to photo-axotomy, there is an antidromic spike in FETi and consequent central EPSP in the flexor. Following axotomy, there is no response in the flexor and only a small, late hyperpolarisation in FETi. The latter may be due to slight movement in the joint region after the muscle has contracted enough to take up the slack.

These experiments show that, when FETi is stimulated with the tibia free to move, the overall depolarising response of the flexor motor neurone is due to summation of the central EPSP with movement-dependent excitatory sensory input, while when the tibia is held fixed in the fully flexed position, the overall response is due to summation of the central EPSP with

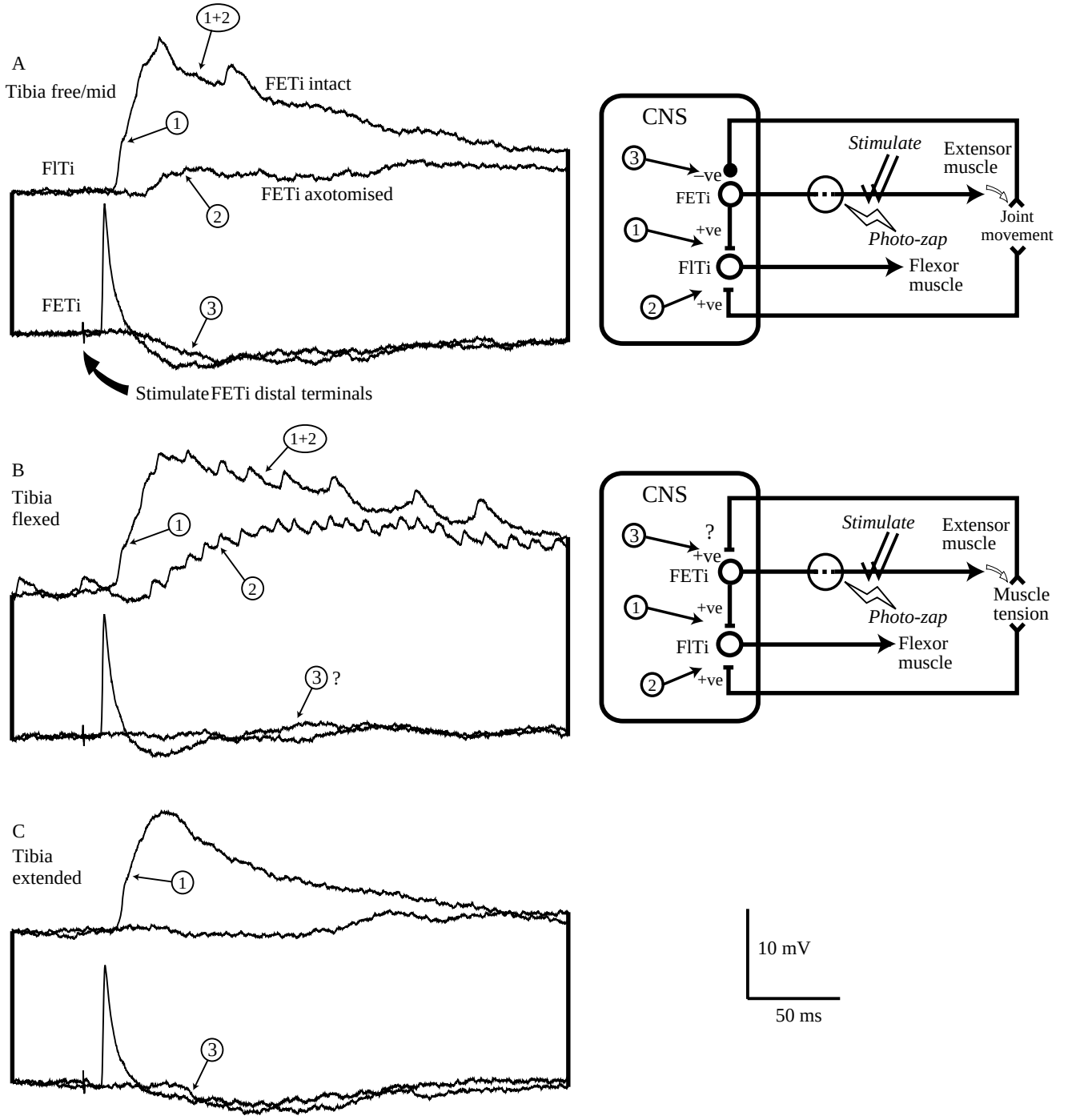


Fig. 2. (A–C) Peripheral reflexes induced by extensor muscle contraction. Each record shows intracellular recordings from a FlTi motor neurone (top traces) and FETi (bottom traces), in response to stimulating the distal FETi axon terminals. Two sweeps are superimposed, one in which the FETi axon is intact (the FETi trace has an antidromic spike, the FlTi trace shows a large initial depolarisation) and the other following FETi photo-axotomy (no antidromic spike in the FETi trace, no large early depolarisation in the FlTi trace). (A) The tibia is initially in the mid position and is free to move in response to the extensor muscle contraction. The cartoon on the right shows the circuit connections operative in these conditions. Prior to axotomy, the antidromic spike in FETi initiates a central EPSP in the FlTi (connection 1), while both prior to and following axotomy the tibial extension movement initiates positive (connection 2) and negative (connection 3) resistance reflexes onto FlTi and FETi respectively. (B) The tibia is held fully flexed. The cartoon shows the relevant circuit connections. Prior to axotomy the antidromic spike in FETi initiates a central EPSP in the FlTi (connection 1), while both prior to and following axotomy the extensor muscle tension initiates positive reflexes onto the FlTi (connection 2) and, more weakly, the FETi (connection 3). The small depolarisations in the FlTi trace are spikes attenuated by passive propagation to the soma recording site. (C) The tibia is held fully extended. Prior to axotomy the antidromic spike in FETi initiates a central EPSP in the FlTi (connection 1), while following axotomy the only effect is a longer-latency inhibitory reflex onto FETi (connection 3, cartoon for A). This effect is also present prior to axotomy, but is partially obscured by the after-hyperpolarisation of the antidromic spike. The records are from one preparation.

extensor tension-dependent sensory input. Both types of excitatory sensory input have a relatively long duration (100–200 ms) and clearly make a major contribution to the second component of the compound flexor EPSP recorded prior to axotomy (Fig. 1). Detailed examination of the timing of the EPSPs before and after FETi axotomy shows that when FETi is activated by antidromic stimulation of its peripheral terminals the movement- and tension-dependent sensory input has a sufficiently short latency to make some contribution to the change in amplitude of the initial component of the compound central–peripheral EPSP (Fig. 3). However, in most cases, the early part of the sensory input has insufficient amplitude to account for the full position-dependent difference in the initial component of the EPSP in the intact preparation, which suggests that there may be a position-dependent influence of the central EPSP independent of phasic sensory input induced by extensor muscle tension. This possibility is explored next.

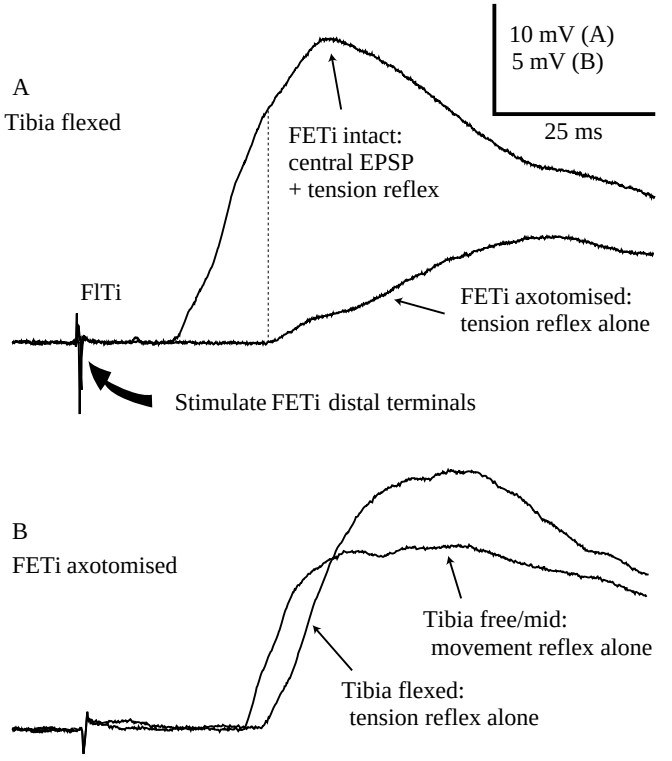


Fig. 3. (A,B) The relative timing of central and peripheral components of the compound EPSP. (A) The distal terminals of FETi are stimulated with the tibia fixed in the flexed position. Two traces are superimposed: before and after FETi axotomy. The early component of the peripheral reflex revealed after axotomy has a short enough latency (dotted line) to affect the peak amplitude of the compound central–peripheral EPSP recorded prior to axotomy. (B) The distal terminals of FETi are stimulated after photo-axotomy. Two traces are superimposed; with the tibia fixed flexed, and with the tibia in the mid position and free to move. The movement-dependent resistance reflex has a shorter latency but lower amplitude than the tension-dependent reflex. The records in A and B are from different preparations. Each trace is the average of eight consecutive responses in which stimuli were applied at 0.5 Hz.

Gain changes induced by tibial position

The role of tibial position in changing the gain of the FETi–flexor central connection was first investigated by cutting the distal tendons of the extensor and flexor muscles in order to reduce the peripheral effects of motor output. Cutting the flexor tendon produced very little difference from the intact preparation (Fig. 4A,B). Cutting the extensor tendon abolished the second component of the flexor response to a FETi spike, but produced only a slight reduction in the position-dependent difference in the first component (Fig. 4C). Under these conditions, the extensor muscle was still contracting but, since it was disconnected from the tibia, it did not produce any movement or cuticle strain. However, it is possible that the movement of the muscle itself might induce some sensory effects, and so the next approach was to photo-axotomise FETi while leaving the peripheral structures within the leg undamaged. Following axotomy, all peripheral effects of the FETi spike were completely abolished, but there was still a substantial position-dependent difference in the amplitude and duration of the EPSP recorded in the flexor motor neurone in response to a FETi spike elicited by current injection. In one preparation (Fig. 4D), the EPSP in the flexor motor neurone had an amplitude of about 10 mV when the leg was held in the fully extended position and about 17 mV when the leg was held in the fully flexed position. We therefore conclude that tibial position by itself can exert a considerable influence on the amplitude of the central EPSP induced in flexor motor neurones by a FETi spike.

The femoral chordotonal organ is a major source mediating the change in gain

The femoral chordotonal organ (FCO) is one of the main sense organs monitoring position and movement of the femoral–tibial joint. Stretch of the tendon of the FCO provides the central nervous system with the information that the leg is flexed, while relaxation of the tendon signals that the leg is extended. Manipulation of the FCO tendon, i.e. alternately stretching and relaxing it, while the leg is continuously held in the extended position, causes changes in both the amplitude and duration of the central EPSP elicited by antidromic stimulation of FETi (Fig. 5). The onset latencies of the EPSPs during stretch and relaxation are virtually identical, but the peak amplitude and width are increased when the FCO tendon is stretched and decreased when it is relaxed. The changes are maintained after photo-axotomy of FETi, when the central EPSP is elicited by injecting brief current pulses into FETi. Thus, the FCO-mediated changes in gain, like those mediated by changes in tibial position, are completely independent of extensor muscle tension or tibial movement (Fig. 5B). These changes are very consistently reproduced in time during alternating periods of sustained stretch and relaxation.

The site and mechanism of position-dependent changes in gain

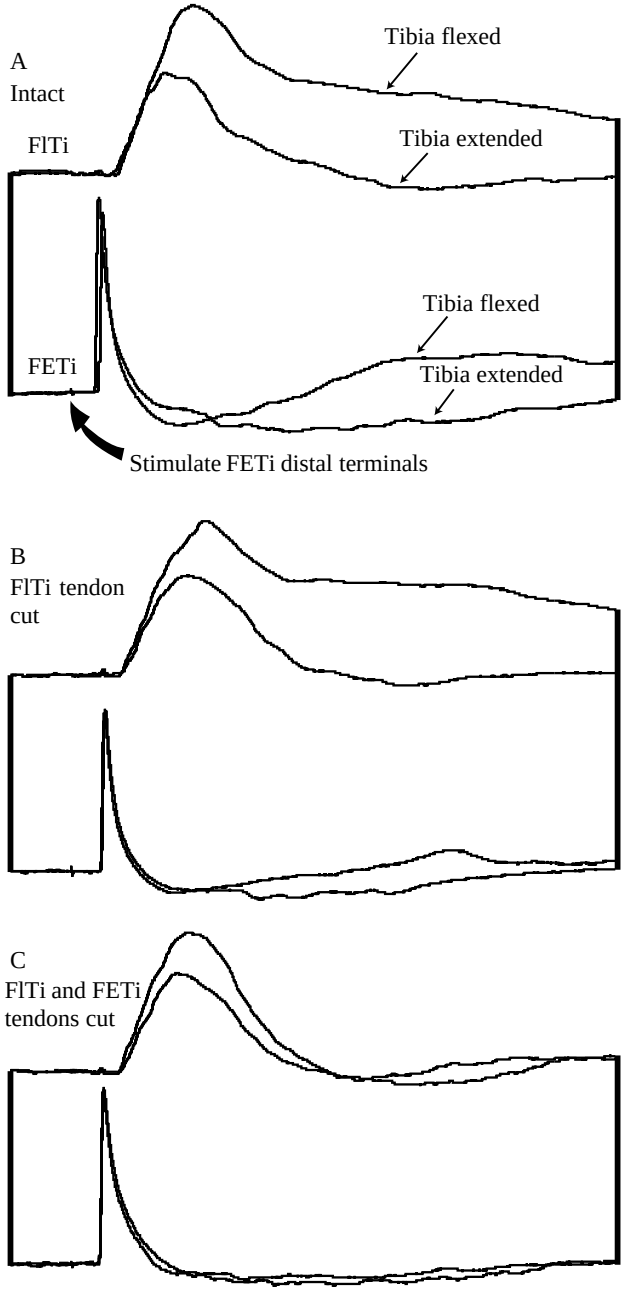
The position-dependent changes in the gain of the FETi–flexor connection could be due to postsynaptic changes

in the flexor motor neurone, presynaptic changes in transmitter release from FETi or a combination of the two.

Postsynaptic mechanisms

If the effect is postsynaptic, two main mechanisms can be envisaged. (1) Tonic excitation of the flexor caused by FCO tendon stretch would sum with the central EPSP and increase its amplitude. (2) Tonic inhibition of the flexor caused by FCO tendon relaxation would short-circuit the EPSP and reduce its amplitude.

If FCO tendon stretch caused a tonic excitation of flexor motor neurones, this could increase the peak depolarisation achieved by the FETi-mediated EPSP, but would reduce rather than increase its relative amplitude (i.e. the amplitude from the immediately preceding baseline membrane potential to the peak). When the baselines are aligned, the data clearly show that the increase in the initial component of the EPSP with tibial flexion (Figs 1, 4) or FCO tendon stretch (Fig. 5A,B) is actually accompanied by an increase in relative amplitude, which suggests that the increase cannot be caused by summation with additional excitatory input. However, tonic depolarisation could increase the number of spikes elicited by the EPSP. The somata of flexor motor neurones are electronically quite distant from spiking membrane, and so in soma recordings from most flexor motor neurones spikes are rather highly attenuated and smoothed. It is therefore possible that an apparent increase in relative EPSP amplitude recorded in the soma might in fact be due to an increase in the number



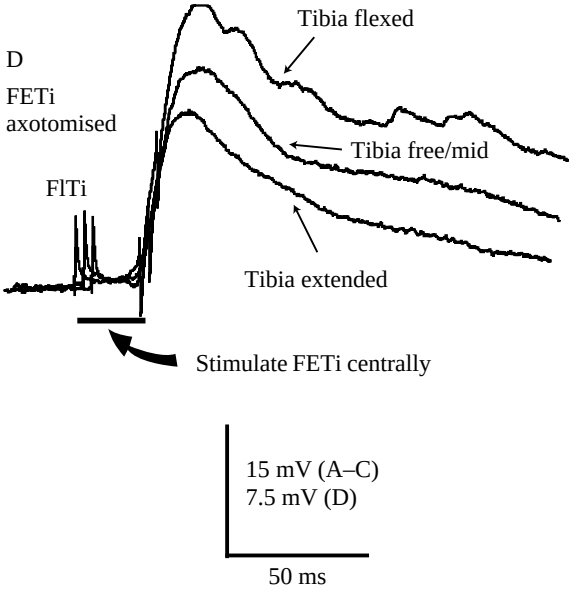
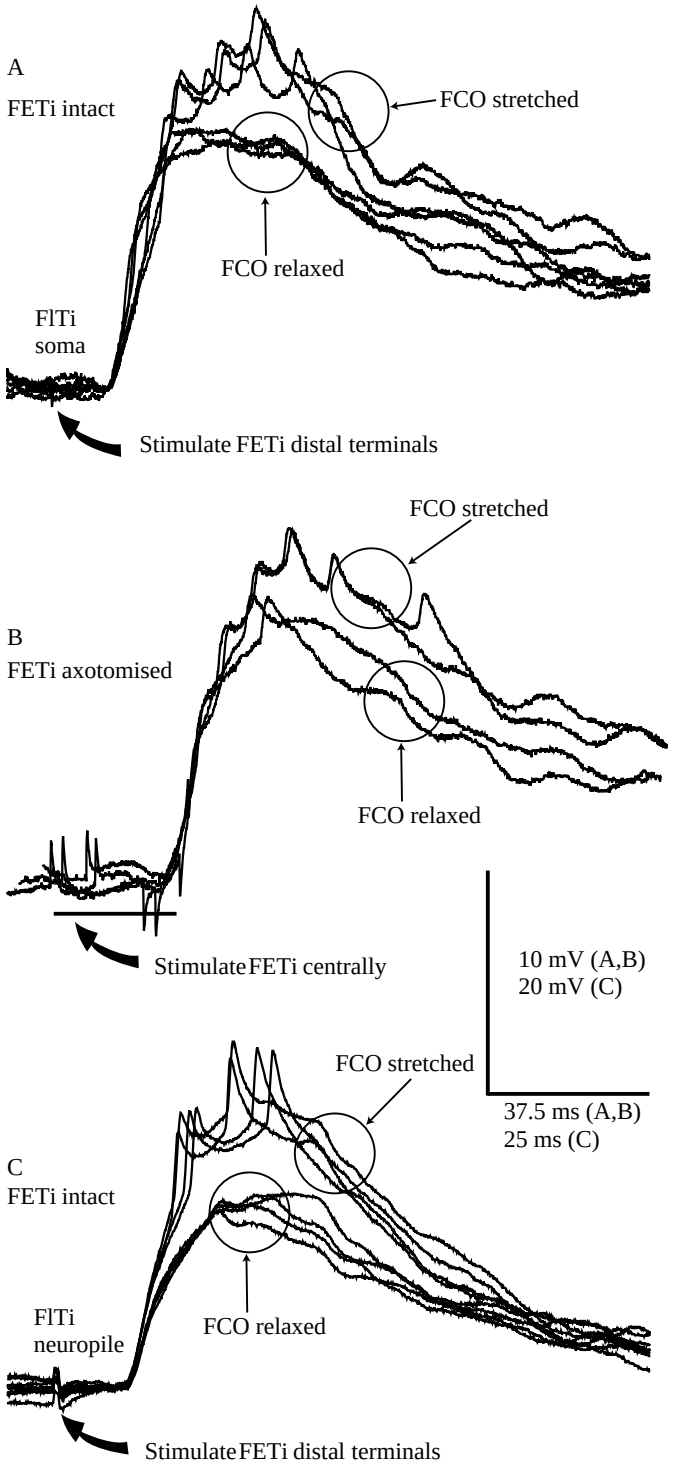


Fig. 4. (A–D) Changes in the amplitude of the central EPSP caused by different leg positions persist after elimination of tension feedback and movement. (A–C) The distal terminals of FETi were stimulated with implanted myogram electrodes, while recording from a FlTi motor neurone (top traces) and FETi (bottom traces). In each record, two sweeps are superimposed, one with the tibia held fully flexed, the other with the tibia held fully extended. (A) In the intact preparation, the amplitudes of both the first and second components of the FlTi response to a FETi spike are changed by tibial position. (B) The change in both components persists after cutting the flexor tendon. (C) The second component is abolished by cutting both the flexor and extensor tendons, but the change in the first component persists. (D) FETi is photo-axotomised. An orthodromic spike in FETi (not shown) is evoked by injecting depolarising current and causes a central EPSP in the FlTi. Since the spike cannot reach the extensor muscle, no muscle tension or leg movement is evoked, but the EPSP in FlTi is still changed by tibial position. Three traces are superimposed, with the tibia fixed flexed, in the mid position and free to move, and fixed in the extended position. The traces are aligned on the rising phase of the EPSP. A–C are from one preparation, D is from another. Each trace is the average of eight consecutive responses in which stimuli were applied at 0.5 Hz.

of spikes elicited by the EPSP, rather than by an increase in the amplitude of the EPSP itself (Burrows *et al.* 1989). To investigate this possibility, recordings were made from the neuropile of flexor motor neurones, where spikes can be clearly distinguished from the underlying EPSP. In six preparations in which recordings were made from the neuropile of flexor motor neurones, the mean spike count per EPSP when the FCO tendon was stretched was 2.2 (range 1–5), while the mean spike count when the tendon was relaxed was 0.5 (range 0–2). However, the recordings clearly show that underlying this change in spike count there is also a substantial change in the amplitude (up to 30 %), duration and rate of rise of the EPSP (Fig. 5C). We therefore think it unlikely that FCO tendon stretch augments the FETi-induced EPSP by summation with tonic postsynaptic depolarisation.



We next consider the possibility that tibial extension or FCO tendon relaxation could diminish the FETi-induced EPSP by summation with postsynaptic inhibition. Moving the tendon of the FCO can induce both dynamic and static sensory input in flexor motor neurones. There is usually a depolarising dynamic response to movement of the FCO tendon in either direction, which is larger when relaxing the tendon (6.7±2.0 mV, N=12) than when stretching it (2.9±1.7 mV, N=12). Sustained stretch or relaxation of the tendon usually causes a relatively small tonic shift in membrane potential (less than 4 mV in soma recordings of flexor), but the direction of this shift varies from one animal to another. Sustained stretch of the tendon was associated with a sustained depolarising shift (1.6±1.3 mV, range 0.5–4.0 mV) in seven animals, with a hyperpolarising shift (0.9±0.4 mV, range 0.4–1.5 mV) in six animals, and with no shift occurring in five animals. In each of these animals, sustained stretch of the FCO tendon increased the amplitude of the FETi-induced EPSP in the flexor motor neurone.

While it is possible that a reduction in the amplitude of the FETi-induced EPSP might have been caused by summation with general postsynaptic inhibition in those preparations where sustained FCO tendon stretch caused tonic flexor hyperpolarisation, this cannot be the explanation in the preparations in which it caused an excitatory depolarisation. In one such preparation, the FETi motor neurone was stimulated continuously at low frequency while applying alternate stretch and relaxation to the FCO tendon and recording from flexor and FETi motor neurones (Fig. 6). The dynamic and static sensory input described above was apparent in the output of the flexor motor neurone, along with the FETi-induced central EPSP. Relaxing the FCO tendon caused an immediate and substantial decrease in both the relative and the absolute amplitude of this specific EPSP, while at the same time eliciting a depolarising excitatory dynamic sensory response in the flexor. Both these effects were maintained, albeit with diminished amplitude, during the period of sustained FCO

Fig. 5. (A–C) Manipulation of the FCO tendon changes the amplitude of the central EPSP with a high degree of consistency. In each record, FETi was stimulated continuously at 0.5 Hz with the tibia held fully extended, while the FCO tendon was alternately stretched and relaxed, with each condition maintained for 40 s. EPSPs were recorded 20 s after each change in FCO tendon position. Superimposed traces are displayed of EPSPs made with the FCO tendon alternately stretched and relaxed. (A) Recordings were made from the soma of a FlTi motor neurone while the distal terminals of FETi were stimulated. The FETi axon was intact. (B) As in A, except that FETi has been axotomised and was stimulated centrally with depolarising current. (C) Recordings were made from the neuropile segment of a FlTi motor neurone while the distal terminals of FETi were stimulated. The FETi axon was intact. Records A and B are from one preparation, C is from another.

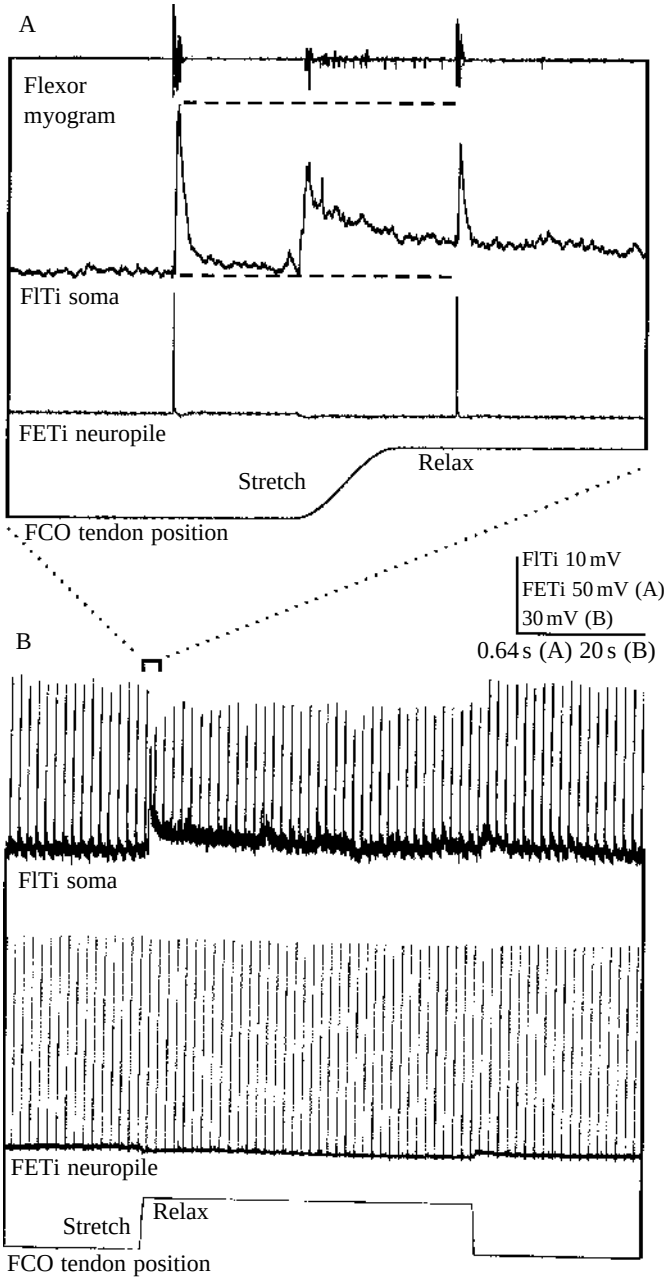


Fig. 6. (A,B) The change in the amplitude of the FETi-induced FlTi EPSP caused by the FCO is not caused by summation with postsynaptic inhibition. The distal terminals of FETi were stimulated continuously at 0.65 Hz while the FCO tendon was alternately stretched and relaxed. (A) Stimulation episodes are shown immediately before and immediately after moving the tendon from the stretched to the relaxed position. The tendon movement (bottom trace) elicits a depolarising reflex in the FlTi motor neurone (second trace, soma recording), which initiates spikes and is therefore excitatory (flexor myogram shown in top trace), and a small hyperpolarising reflex in the FETi (third trace, neuropile recording). Following tendon relaxation, the FlTi EPSP induced by the FETi spike arises from a depolarised baseline (dashed line), but is reduced in amplitude (dashed line) compared with the EPSP resulting from the preceding FETi stimulation episode. (B) An extended record showing a complete stretch–relax–stretch cycle of FCO tendon movement, including the relaxation shown in A (bracket). Traces as in A, but without the flexor myogram.

tendon relaxation and were reversed upon subsequent tendon stretch. Thus, FCO tendon relaxation caused a diminution in the amplitude (both relative *and* peak) of the FETi-induced EPSP in a situation where the flexor motor neurone was receiving excitation, not inhibition. We therefore regard it as unlikely that FCO tendon relaxation diminishes the FETi-induced EPSP by summation with general tonic postsynaptic input.

Presynaptic mechanisms

Variations in the amplitude of FETi spikes recorded in the soma can often be correlated with variations in the amplitude of the flexor EPSP (Heitler and Burrows, 1977*b*; Burrows *et al.* 1989), and relaxing the FCO tendon invariably causes a reduction of 15–20 % in FETi spike amplitude during the actual movement, while at the same time reducing the amplitude of the FETi-induced EPSP in flexor motor neurones. However, this major change in FETi spike amplitude which occurs during the tendon movement is not maintained after the tendon movement terminates, while the change in EPSP amplitude is maintained, albeit at a reduced level (Fig. 6). We examined the amplitude and waveform of antidromic spikes recorded from the soma and from the main neuropile segment of FETi, to look for any tonic changes in spike characteristics associated with maintained changes in FCO tendon position. We could not detect any difference at either recording site in the relative amplitude (i.e. from baseline to peak) or shape between FETi spikes which elicited large-amplitude EPSPs in the flexor with the FCO tendon stretched and spikes which elicited smaller-amplitude EPSPs with the FCO tendon relaxed (Fig. 7). However, we did find that there was a small increase in the absolute peak voltage of the spike when the FCO tendon was stretched (19.55±0.50 mV, N=132 spikes) compared with that when the tendon was relaxed (19.11±0.52 mV, N=123 spikes), which correlated with the change in flexor EPSP (Fig. 8). The change in peak amplitude also correlated almost exactly with, and was probably caused by, a slight tonic difference in resting FETi membrane potential (Fig. 6; FCO stretched −57.4±0.51 mV, N=132; FCO relaxed −58.1±0.53 mV, N=123). Although small, the differences in both spike peak and membrane potential were statistically highly significant (t-test, P<0.001). Similar changes in membrane potential were observed in 17 preparations (mean difference 0.85 mV depolarised stretched relative to relaxed). Two other preparations showed no change in FETi membrane potential with stretch of the FCO tendon and none showed a hyperpolarisation with stretch. No change in spike width was apparent between the two tendon positions in any preparation.

The changes in FETi spike caused by FCO tendon position are small, and the correlation with the changes in flexor EPSP may not necessarily be causal to them. We therefore attempted to manipulate the FETi spike independently of any other factor. FETi was stimulated antidromically from its peripheral terminals and the resulting EPSP was recorded in a flexor motor neurone. The same antidromic stimulus was then applied while a pulse of negative current was injected into the

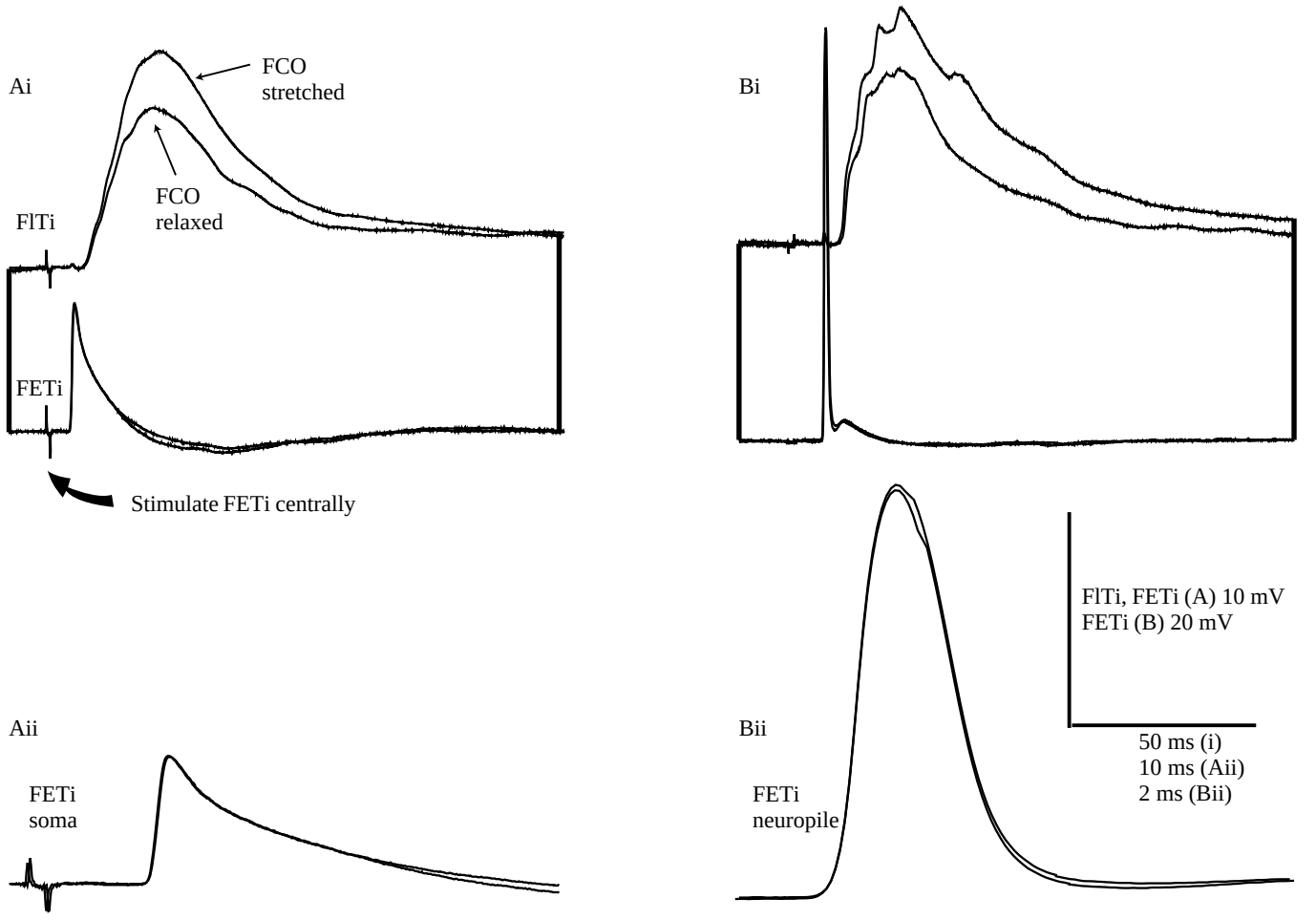


Fig. 7. (A,B) There is no major change in the shape of the FETi spike correlated with the changes in the amplitude of the FlTi EPSP. In each record, FETi was stimulated continuously at 0.5 Hz with the tibia held fully extended, while the FCO tendon was alternately stretched and relaxed, with each condition maintained for 40 s. Recordings were made 20 s after each change in FCO tendon position. (Ai) Recordings were made from a FlTi motor neurone (top traces) and the soma of FETi (bottom traces). (Bi) As in A, except FETi was recorded in the neuropile. (Aii,Bii) Expanded sections of the FETi traces shown in Ai and Bi. Each trace is the average of eight stimulation episodes. A and B are from different preparations.

neuropile of FETi, timed so as to bracket the antidromic spike. The EPSP recorded in the flexor when FETi was injected with negative current was reduced in both amplitude and duration compared with the EPSP recorded when no current was injected (Fig. 9). This shows that experimentally induced presynaptic changes in FETi can produce changes in the postsynaptic response.

Discussion

The metathoracic fast extensor tibiae (FETi) motor neurone of the locust is highly unusual in that it is the only known example of an insect motor neurone which makes chemical synaptic output within the central nervous system as well as peripheral output to a muscle. It has a strong monosynaptic excitatory connection to most if not all of the antagonist flexor tibiae motor neurones. The amplitude and shape of the EPSP induced in the flexors in response to a spike in FETi are dependent on the peripheral tibial condition. If the FETi spikes with the tibia held flexed, the EPSP is significantly larger than if FETi spikes with the tibia free to move or held extended. This difference in EPSP amplitude can cause a significant difference in the number of flexor motor spikes which are produced and, hence, in the flexor muscle tension. The change is therefore likely to affect the behavioural output of the system.

There are two separate mechanisms by which the peripheral conditions can alter the amplitude of the central FETi-induced flexor EPSP. The first is that the extensor muscle contraction resulting from the FETi spike can cause sensory responses which feed back to the flexor motor neurones and sum with the central EPSP. The form of the sensory feedback depends upon the peripheral condition. When the tibia is free to move, an extension-dependent depolarisation adds to the central EPSP (Fig. 2A). When the tibia is held in a fixed position, a tension-dependent depolarisation, whose magnitude strongly depends

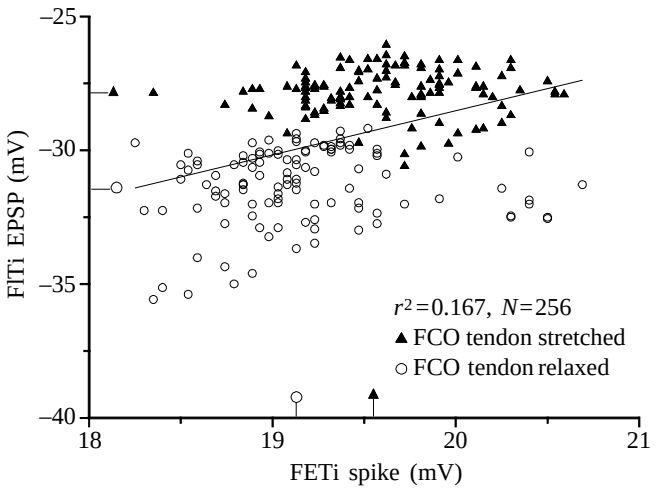


Fig. 8. Graph showing that peak FETi neuropile spike amplitude is correlated with peak FlTi EPSP amplitude measured in the soma and that both are dependent on FCO tendon position. FETi was stimulated continuously at 0.65 Hz, and the FCO tendon was moved between the stretched and relaxed positions approximately every 40 s. Data from six stretched and relaxed episodes are shown. Spike–EPSP pairs occurring within 5 s of FCO tendon movement are not included, so the data represent static changes only, with the augmented dynamic changes excluded. The line shows the best-fitting linear regression through the data points, and the correlation coefficient r^2 is given. The locations of the mean values are indicated on the axes by short lines terminating in the appropriate symbol. See text for statistical comparison of means

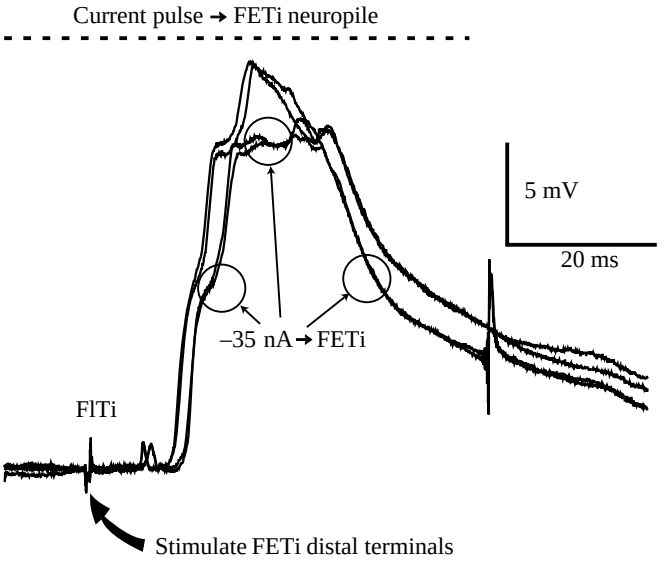


Fig. 9. The FETi-induced EPSP in a FlTi motor neurone can be reduced in amplitude and duration by injecting negative current into the neuropile of FETi. Each trace is the average of eight consecutive responses. The distal terminals of FETi were continuously stimulated at 0.5 Hz. In two traces, the FETi spike was bracketed by a pulse of 35 nA of negative current (dotted line shows pulse, which started before the displayed trace), while in the other two traces no current was injected. Trace averages with and without current were recorded alternately.

on leg position, adds to the central EPSP (Fig. 2B,C). This mechanism is quite simple; it merely involves the summation of the central EPSP with peripheral input. The second mechanism is that the tibial position at the time of the FETi spike can itself alter the central EPSP irrespective of tension- or movement-dependent feedback. This has been demonstrated both by cutting the flexor and extensor muscle tendons, thereby eliminating tibial movement and cuticular stress in response to extensor muscle contraction, and also by specific photo-axotomy of the FETi, thereby eliminating the muscle contraction itself. This latter procedure prevents the FETi from activating the extensor muscle, but does not damage the central FETi–flexor connection, nor does it alter the position-dependent feedback from position-sensing proprioceptors which modulates the flexor EPSP. The manner in which the second mechanism operates is discussed next.

The mechanism of the position-dependent changes in gain

The simplest way for the tibial position to alter the gain of the FETi–flexor connection would be if the FCO were to mediate general tonic sensory input onto the flexor which summed with the central EPSP to change its amplitude (which is essentially the same principle as for the movement- and tension-dependent effects). However, this does not appear to be what happens. First, we consider the case of tibial extension or FCO tendon relaxation. This condition reduces the amplitude of the FETi-induced EPSP, but frequently depolarises the flexor motor neurones (as would be expected for a simple resistance reflex) and can make them spike (Fig. 6). The reduction in EPSP amplitude cannot in these cases, therefore, be caused by concurrent general inhibition of the flexor motor neurone, since no such inhibition occurs. Could the conductance increase associated with the tonic excitation 'shunt' the FETi-induced EPSP so as to reduce its amplitude? The data show (Fig. 6) that the absolute peak amplitude of the FETi-induced EPSP is reduced when summed with the tonic excitation. For this reduction to be a result of shunting would require that the tonic excitation had a reversal potential which was more negative than the voltage normally attained by the FETi-induced EPSP on its own, but still above spike threshold, and this seems unlikely. We thus conclude that the reduction in the amplitude of the FETi-induced EPSP with tendon relaxation is unlikely to be due to postsynaptic summation with tonic input, either inhibitory or excitatory. Next, we consider the case of tibial flexion or FCO tendon stretch, which increases the amplitude of the FETi-induced EPSP. If this increase were due to summation with tonic excitation, the peak amplitude of the FETi-induced EPSP would indeed be increased, but the relative amplitude (from the immediately preceding depolarised baseline) would be decreased because of the closer approach to reversal potential. The data clearly show (Figs 1, 4, 5, 7) that the relative, as well as the absolute, peak amplitude of the FETi-induced EPSP is increased, and therefore the change is unlikely to be caused by

postsynaptic summation with tonic excitatory input to the flexor.

These results seem to point in the direction of a presynaptic mechanism for the change in amplitude of the flexor EPSP. It is known that a correlation exists between changes in FETi spike amplitude and changes in the shape of the flexor EPSPs (Heitler and Burrows, 1977*b*; Burrows *et al.* 1989), but these have not been related to tibial position. Furthermore, pharmacological manipulations (application of 5-hydroxytryptamine) which increase the width of the FETi spike can substantially increase the amplitude of the flexor EPSP (Parker, 1995), and this constitutes a plausible mechanism for mediating the changes in FETi–flexor gain. However, we have not been able to detect any change in the relative shape of the antidromic spike which correlates with the change in EPSP amplitude and which is caused by changes in the FCO tendon position, when recording either from the soma or the neuropile (Fig. 7). We have found a small (<1 mV) but statistically significant change in the FETi membrane potential and peak spike amplitude brought about by FCO tendon manipulation; the spike achieves a more depolarised peak when the FCO tendon is stretched than when it is relaxed, and this fits with the direction of the change in amplitude of the flexor EPSP. Further indirect supporting evidence for a presynaptic mechanism derives from the effects of passing hyperpolarising current into the FETi neuropile while evoking FETi spikes (Fig. 9). Spikes which were bracketed by negative current consistently evoked flexor EPSPs of reduced amplitude and duration. The level of current required to produce this effect was quite large, but the current was injected into the soma, and therefore there would have been considerable attenuation during propagation to the active regions. Of course, injection of current into FETi is not an accurate mimic of the putative modulation of FETi brought about by synaptic events, but this result, combined with the pharmacological data (Parker, 1995), does demonstrate the feasibility of the proposition that the postsynaptic flexor response can be modulated by presynaptic events within FETi.

Position-dependent gain control may be localised to the FETi–flexor connection

The changes recorded in presynaptic membrane potential and spike amplitude with tibial position, although significant, are very small. Either these changes are genuinely very small, in which case they almost certainly cannot account for the changes in the EPSP, or else they reflect larger changes occurring a long electrotonic distance from the recording site. The FETi has a large cell body and thick primary neurite (where neuropile recording electrodes are placed), but the secondary neurites which mediate much of the synaptic connection to flexor motor neurones are almost all of very small diameter (see Fig. 1 in Watson and Burrows, 1982). This means that any localised perturbation in either membrane potential or spike shape produced by modulating synaptic input occurring on the presynaptic terminals of FETi would be highly attenuated by the time it propagated to either a somatic or neuropile recording site. A similar argument applies to the possibility of localised postsynaptic modulation. Although we discount the possibility of a generalised postsynaptic mechanism, there could be highly specific modulation of the flexor at the sites of input from the FETi connection. Thus, FCO tendon relaxation could cause localised inhibition of these input sites while at the same time causing an excitation at other sites closer to the flexor spike-initiating sites and therefore exciting the flexor as a whole. The hypothesis of localised modulation is supported by the existence of unidentified synaptic elements which make common inputs onto both FETi and flexor motor neurones, many of which are located within 1 μm of the FETi–flexor synaptic contact sites (Burrows *et al.* 1989). Thus, while our data do not allow us to distinguish definitively between pre- and postsynaptic mechanisms for the changes in gain, they do suggest that the effect is specific and targeted directly at the FETi–flexor connection, and thus can be reasonably described as a modulation of this synapse.

The femoral chordotonal organ is a major source of position-dependent gain control

The femoral chordotonal organ (FCO) is a major source of the position-dependent change in the gain of the FETi–flexor connection, since stretching and relaxing the tendon of the FCO, while keeping the tibia in a fixed extended position, can produce changes in gain which are similar in amplitude to those seen in intact animals (Figs 5, 6). Other sensory systems which monitor tibial position, such as the flexor strand of the FCO or the joint receptors with axons in the lateral nerve (Coillot and Boistel, 1968), do not make a major contribution to the changes in gain, since in these experiments the fixed position of the tibia would also have fixed their output. However, it is possible that in the intact animal they may make some additional contribution to the gain control.

Functional significance of peripheral control of the gain of the central synapse

At first sight, the FETi–flexor excitatory connection seems highly maladaptive, since the hard-wired activation of one muscle concurrent with the activation of its antagonist would seem inappropriate in most circumstances. However, the metathoracic FETi motor neurone is highly specialised and is mainly used in two types of motor programme. The first is the jump/kick/swim motor programme (Heitler and Burrows, 1977*a*; Pflüger and Burrows, 1978) and the second is 'thrusting'. The peripheral control of the gain of this central connection that we report in this paper fits well with the different functional requirements of these two behaviours.

The jump/kick/swim motor programme is a three-stage event. In the first stage, flexor motor neurones are active to bring the tibia into the fully flexed position. In the second stage, both flexor motor neurones and the FETi motor neurone are active. The flexor muscles keep the tibia flexed, while the very strong extensor muscle builds up tension almost isometrically and stores energy by distorting elastic cuticular elements. In the third stage, the flexor motor neurones are strongly inhibited

to release the tibia, which extends using the stored elastic energy. Thus, in this motor programme there is indeed a functional requirement for co-activation of flexor and extensor motor neurones. In fact, it is essential that the flexors should have a high level of activity as soon as the FETi motor neurone starts to spike, since if the level of flexor tension is inadequate, the extensor muscle will exert greater torque than the flexor, and the tibia will extend before the extensor muscle has stored sufficient energy to ensure the success of the behaviour. However, the second stage co-activation of FETi and flexors only occurs once the tibia has reached the fully flexed position (Bässler, 1967; Usherwood *et al.* 1968; T. Jellema and W. J. Heitler, in preparation), and therefore at the very moment when the functional requirement for the FETi–flexor excitatory connection is maximal, the peripheral gain control mechanism which we describe will augment its amplitude.

Thrusting is a defensive behaviour which occurs if the tibia meets an obstacle during an attempted extension. Cuticle strain detectors in the proximal tibia mediate positive excitatory feedback onto FETi which can lead to repetitive FETi spiking as the animal pushes against the obstacle (Burrows and Pflüger, 1988). In this behaviour, concurrent activation of flexor motor neurones by the FETi–flexor central connection would indeed be maladaptive, since presumably it would reduce the net extension force. However, thrusting usually occurs with the tibia partially extended, and so the position-dependent peripheral gain control which we describe would reduce the amplitude of the FETi-induced EPSP and thereby reduce its maladaptive effect in these circumstances.

In conclusion, this paper shows how peripheral sensory input can control the gain of a hard-wired central synaptic connection so as to augment its effectiveness during a motor programme in which it is beneficial and to reduce its effectiveness during a motor programme in which it would be maladaptive.

This work was supported by a grant from the Biotechnology and Biological Sciences Research Council (formerly Science and Engineering Research Council) of the United Kingdom.

References

BÄSSLER, U. (1967). Zur Regelung der Stellung des Femur–Tibia-Gelenkes bei der Stabheuschrecke *Carausius morosus* in der Ruhe und im Lauf. *Kybernetik* **4**, 18–26.

BENNET-CLARK, H. C. (1975). The energetics of the jump of the locust *Schistocerca gregaria*. *J. exp. Biol*. **63**, 53–83.

BURROWS, M. (1987). Parallel processing of proprioceptive signals by spiking local interneurons and motor neurons in the locust. *J. Neurosci*. **7**, 3282–3292.

BURROWS, M. (1988). Responses of spiking local interneurones in the locust to proprioceptive signals from the chordotonal organ. *J. comp. Physiol.* A **164**, 207–217.

BURROWS, M. AND MATHESON, T. (1994). A presynaptic gain control mechanism among sensory neurons of a locust leg proprioceptor. *J. Neurosci.* **14**, 272–282.

BURROWS, M. AND PFLÜGER, H. J. (1988) Positive feedback loops from proprioceptors involved in leg movements of the locust. *J. comp. Physiol.* A **163**, 425–440.

BURROWS, M., WATSON, A. H. D. AND BRUNN, D. E. (1989). Physiological and ultrastructural characterization of a central synaptic connection between identified motor neurons in the locust. *Eur. J. Neurosci.* **1**, 111–126.

COILLOT, J. P. AND BOISTEL, J. (1968). Localisation et description de recepteurs a l'étirement au niveau de l'articulation tibio-femorale de la patte sauteuse du criquet *Schistocerca gregaria*. *J. Insect Physiol.* **14**, 1161–1667.

FIELD, L. H. AND BURROWS, M. (1982). Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. *J. exp. Biol*. **101**, 265–285.

HEITLER, W. J. (1978). Coupled motoneurons are part of the crayfish swimmeret central oscillator. *Nature* **275**, 231–234.

HEITLER, W. J. (1995). Quasi-reversible photo-axotomy used to investigate the role of extensor muscle tension in controlling the kick motor programme of grasshoppers. *Eur. J. Neurosci*. **7**, 981–992.

HEITLER, W. J. AND BURROWS, M. (1977*a*). The locust jump. I. The motor programme. *J. exp. Biol*. **66**, 203–219.

HEITLER, W. J. AND BURROWS, M. (1977*b*). The locust jump. II. Neural circuits of the motor programme. *J. exp. Biol*. **66**, 221–241.

HOYLE, G. AND BURROWS, M. (1973). Neural mechanisms underlying behavior in the locust *Schistocerca gregaria*. I. Physiology of identified motor neurones in the metathoracic ganglion. *J. Neurobiol.* **4**, 3–41.

MILLER, J. P. AND SELVERSTON, A. I. (1979). Rapid killing of single neurons by irradiation of intracellularly injected dye. *Science* **206**, 702–704.

MULLONEY, B. AND SELVERSTON, A. I. (1974). Organisation of the stomatogastric ganglion of the spiny lobster. I. Neurons driving the lateral teeth. *J. comp. Physiol*. **91**, 1–32.

PARKER, D. (1995). Serotonergic modulation of locust motor neurons. *J. Neurophysiol*. **73**, 923–932.

PFLÜGER, H.-J. AND BURROWS, M. (1978). Locusts use the same basic motor pattern in swimming as in jumping and kicking. *J. exp. Biol.* **75**, 81–93.

RENSHAW, B. (1940). Activity in the simplest spinal reflex pathways. *J. Neurophysiol*. **3**, 373–387.

SKORUPSKI, P. AND SILLAR, K. T. (1988). Central synaptic coupling of walking leg motor neurones in the crayfish: implications for sensorimotor integration. *J. exp. Biol.* **140**, 355–379.

USHERWOOD, P. N. R., RUNION, H. I. AND CAMPBELL, J. I. (1968). Structure and physiology of a chordotonal organ in the locust leg. *J. exp. Biol*. **48**, 305–323.

WATSON, A. H. D. AND BURROWS, M. (1982). The ultrastructure of identified locust motor neurones and their synaptic relationships. *J. comp. Neurol.* **205**, 383–397.