

## REFLEX EFFECTS OF THE FEMORAL CHORDOTONAL ORGAN UPON LEG MOTOR NEURONES OF THE LOCUST

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### SUMMARY

The femoral chordotonal organ (FCO) in a hind leg of a locust monitors the position and movement of the tibia about the femur. It consists of a group of sensory neurones embedded in connective tissue attached distally by two structures: the *apodeme*, which inserts close to the apodeme of the extensor tibiae muscle, and the *flexor strand*, which inserts at the base of the apodeme of the flexor tibiae muscle. The action of the apodeme and the flexor strand is reciprocal during movements of the tibia; the apodeme is stretched during flexion of the tibia whilst the flexor strand is relaxed. During extension, the apodeme is relaxed and the flexor strand is stretched. To analyse the reflex effects of this sense organ, all other sense organs of a hind leg were denervated. The apodeme of the FCO was then grasped between forceps, severed from its distal attachment site and its movements controlled by a function generator. The flexor strand remained intact and could be stimulated independently by moving the tibia.

The different reflex effects mediated by the separate stimulation of the two components of the FCO were revealed by making intracellular recordings from the somata of leg motor neurones in the metathoracic ganglion. A movement stimulus to either component in a way that corresponded to tibial extension, excited flexor tibiae and inhibited extensor tibiae motor neurones. There was also an inter-joint effect whereby extension excited the depressor tarsi and inhibited the levator tarsi motor neurones. A flexion movement had the converse effects on these motor neurones.

The effectiveness of the two components was dependent upon the velocity of the stimulus, the set position of the femoro-tibial joint at which the stimulus was applied, the initial direction of movement, and the activity of other neurones in the central nervous system. Slow motor neurones were depolarized more by low velocities of movement, whereas fast ones were depolarized more by high velocities. The two components produced their greatest effects at the set positions where they were most stretched; thus the apodeme was most effective when the joint was flexed, and the flexor strand when it was extended.

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Elicited movements of the hind legs or apparently spontaneous changes of excitability enhanced or masked the typical response of the motor neurones to stimulation of the FCO, indicating that the effects of this sense organ are not to be viewed as rigid, but as modifiable in the context of the behaviour of the animal.

#### INTRODUCTION

The most intensively studied proprioceptor in insects is the femoral chordotonal organ in the legs. This organ consists of a group of sensory neurones embedded in connective tissue which monitor the position of the tibia about the femur, and respond to movements at the femoro-tibial joint (locust: Usherwood, Runion & Campbell, 1968; Burns, 1974; grasshopper: Moran, Varela & Rowley, 1977; stick insect: Bässler, 1965). These sensory neurones contribute to resistance reflexes in the muscles which move the tibia (Usherwood *et al.* 1968; Burns, 1974; Bässler, 1977). However, movement of the tibia about the femur also excites other receptors so that it is difficult to attribute an observed motor effect to a particular sense organ (Hoyle & Burrows, 1973; Burrows & Horridge, 1974; Burrows, 1979; Pearson, Heitler & Steeves, 1980; Siegler, 1981*a, b*).

Therefore to assess the role of the femoral chordotonal organ itself in controlling movements of the legs during posture and locomotion, it is necessary to activate this sense organ independently from all others. Intracellular recording in the central nervous system would then reveal the synaptic potentials as well as the spikes evoked by the chordotonal organ in the leg motor neurones. We have used such methods here to describe the synaptic input to tibial and tarsal motor neurones as a result of mechanical manipulation of the chordotonal organ. These imposed movements corresponded to the natural movements of the femoro-tibial joint during posture and locomotion. It is shown that the effects in the motor neurones are dependent upon the set position of the joint and the initial direction of movement. The effects upon a particular motor neurone form recognizably repeatable patterns that are nevertheless subject to considerable change correlated with changes in other central neurones.

#### MATERIALS AND METHODS

All experiments were performed on adult male and female locusts, *Schistocerca americana gregaria* (Dirsh) (= *S. gregaria*, Forskal) obtained from our own crowded culture. A locust was mounted on its back in plasticene, with all joints of its legs restrained except the femoro-tibial and tibio-tarsal joints of the left hind leg.

The femoral chordotonal organ (FCO) of the left hind leg was exposed by removing a window of cuticle from the lateral (anterior) and ventral sides of the femur, distal to the origin of the accessory flexor tibiae muscle. The highly tanned cuticle of the dorsal ridge of the femur was reinforced with dental plastic (Scutan, Espe, Seefeld, F.R.G.) to prevent fracture when the flexor tibiae muscle developed force. Overlying tracheae were removed, as well as the lateral accessory flexor tibiae muscle which lies parallel to one of the distal attachments of the FCO, the flexor strand. Nerves 5B1 (distal to the FCO), 5B2 (numbering after Campbell, 1961), the lateral

nerve (Heitler & Burrows, 1977), and the cuticular sensory nerves running distally from the FCO were all cut, limiting the afference elicited by tibial movement to that from the FCO. The main distal attachment of the FCO apodeme (Fig. 1) was moved by grasping it between the tips of a pair of shortened forceps that were in turn connected to an electromagnetic vibrator (Ling model 101). Movements of the vibrator were controlled by a function generator. Before dissection of the apodeme, the angle of the femoro-tibial joint was always set at  $130^\circ$  and the function generator set to a precalibrated position. After attaching the forceps, the apodeme was cut distal to the forceps, thereby creating an open loop driven by the function generator. The absolute position and amplitude of movement of the tips of the forceps could then be determined from the curve relating longitudinal displacement of the apodeme against the equivalent angle of the femoro-tibial joint (Fig. 1). Recordings from the FCO nerve showed that movement of the forceps at frequencies as high as 40 Hz adjacent to but not touching the FCO did not stimulate the FCO by vibration transmitted through the saline. This indicates that stimulation of the FCO apodeme did not cause simultaneous stimulation of the flexor strand.

The FCO flexor strand was left intact and could be moved independently of the FCO apodeme by tying the tibia to a rod extending from a second vibrator driven by a second function generator. Rotational movements of the tibia about the femur were measured against a miniature protractor mounted behind the femoro-tibial joint.

'Equivalent angular' movement or position refers to the manipulation of the FCO apodeme. 'Joint' movement or position refers to manipulation of the flexor strand by moving the tibia. Throughout, the stimulus amplitude and velocity are described in terms of equivalent angular movement of the FCO apodeme, or joint movement driving the flexor strand, and the frequency of the applied waveform. The 'set position' is the equivalent angular position or joint angle at which the movement commenced. During stimulation of one FCO component, the other was always held static at  $60\text{--}70^\circ$ .

Intracellular recordings were made from the somata of motor neurones in the metathoracic ganglion (see Hoyle & Burrows, 1973). The anterior and posterior connectives of this ganglion and its lateral nerves, except some branches of nerve 2, remained intact. More consistent reflex effects were found in some experiments if the anterior connectives, and nerves other than nerve 5, were pinched. The tracheal supply to the ganglion was maintained so that ventilation could provide an adequate supply of oxygen. The ganglion was stabilized on a wax-coated stainless-steel platform and the thorax was bathed with a constant flow of saline at  $19\text{--}20^\circ\text{C}$ . The sheath of the ganglion was treated with a 1% (w/v) solution of protease (Sigma type VI) in saline for 2 min. Microelectrodes were filled with potassium acetate and had d.c. resistances of  $30\text{--}50\text{ M}\Omega$  in saline. Recordings from the FCO nerve were made with glass suction electrodes. Pairs of  $50\text{ }\mu\text{m}$  wires insulated except at their tips were used to record from muscles in a hind leg.

To characterize the input to a motor neurone the following sequence of stimuli was presented to the FCO apodeme at 1 min intervals and then repeated for the flexor strand. First, a triangular waveform representing a  $20^\circ$  movement of the femoro-tibial joint was presented at three standard positions of the joint, or equivalent angular

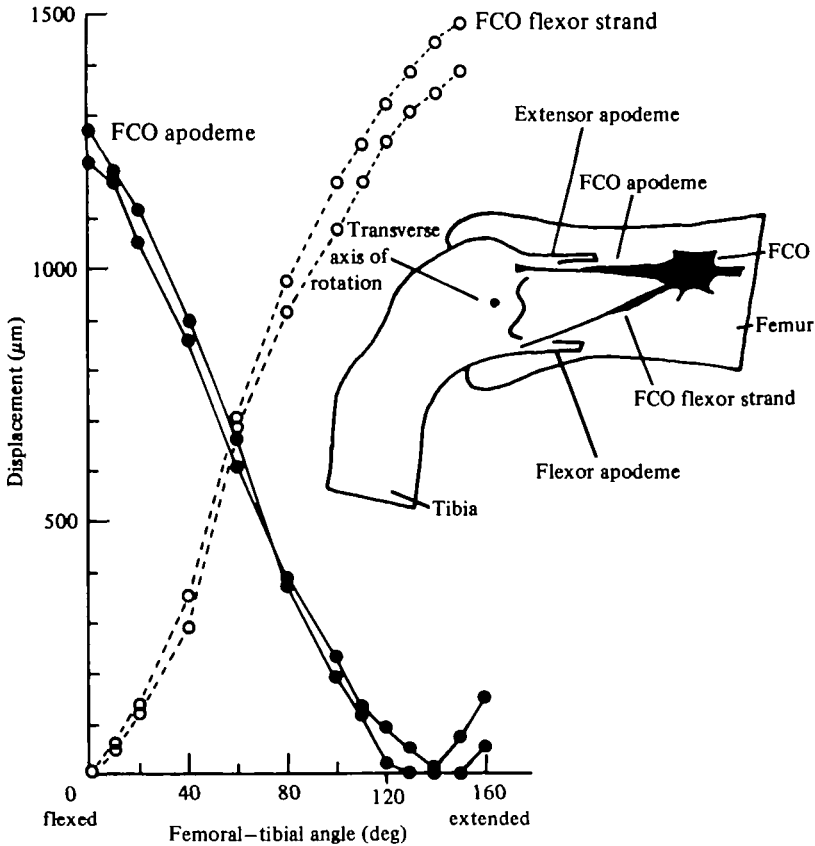


Fig. 1. Relationship between the movement of the two components of the FCO and the angle of the femoro-tibial joint. The movements of the apodeme (●) and the flexor strand (○) were determined by measuring the positions (along the longitudinal axis of the leg) of their distal attachments (relative to a fixed point on the femur) with an ocular micrometer in a dissecting microscope, whilst setting the femoro-tibial joint at different angles. The curves are from two animals. The inset shows the anatomical arrangement of the joint, the chordotonal organ and the apodemes of the flexor and extensor tibiae muscles.

positions (extended =  $130-110^\circ$ , mid =  $90-70^\circ$ , flexed =  $50-30^\circ$ ). Secondly, the  $20^\circ$  stimulus was delivered at frequencies between 0.1 and 20 Hz at the position where it had previously elicited the greatest response. Thirdly, a repetitive triangular movement at 5 Hz was delivered at this same position for at least 10 s. The results are based on 104 successful intracellular recordings from motor neurones in 41 locusts.

## RESULTS

### *Displacement of the FCO*

The FCO is attached proximally to the hypodermis on the distal, outer (anterior) face of the femur. The distal end of the FCO is connected to the femoro-tibial joint by two structures (Fig. 1): (a) the cuticular *FCO apodeme* which attaches to a small protuberance lateral to the insertion of the apodeme of the extensor tibiae muscle, and (b) the *FCO flexor strand*, a hyaline strand which emerges from a spindle-shaped

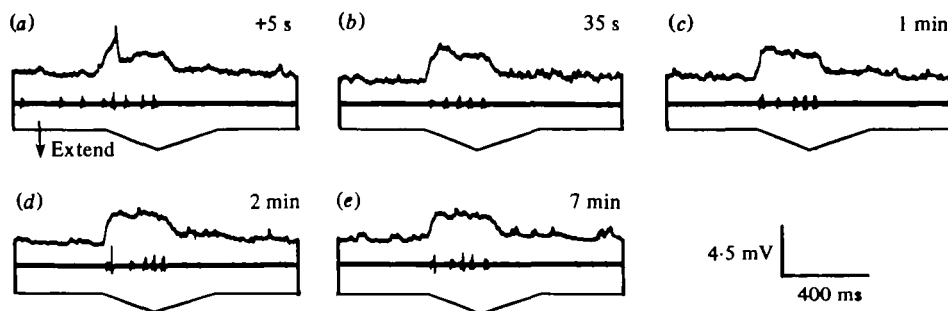


Fig. 2. Test for the possible effects of maintained angle of the femoro-tibial joint on subsequently imposed phasic movements of that joint. The joint was moved from  $130^\circ$  to  $30^\circ$  and then at intervals up to 7 min after this was moved in a triangular function from  $30^\circ$ – $50^\circ$ – $30^\circ$ . Five examples (a–e) of the effect after different intervals upon the membrane potential of a posterior fast flexor tibiae motor neurone (upper trace) are shown. The flexor tibiae myogram is on the middle trace. A movement corresponding to extension of the tibia is indicated by a downward deflexion of the lower trace. This convention is maintained in subsequent figures. There is no change in responsiveness at intervals greater than 5 s after the maintained change of angle.

cluster of sensory neurones of the FCO and broadens to attach at the base of the apodeme of the flexor tibiae muscle. These two connexions have a reciprocal action when the femoro-tibial joint is moved. During extension of the tibia, the FCO apodeme is relaxed, becoming buckled at extreme extension, and the flexor strand is stretched (Fig. 1). During flexion of the tibia, the FCO apodeme is stretched and the highly elastic FCO flexor strand is relaxed but never becomes buckled. Movement of the distal attachment site of the flexor strand follows a sigmoidal curve between  $0^\circ$  (fully flexed) and  $150^\circ$  (fully extended) (Fig. 1). The displacement of the FCO apodeme is approximately linear from 0 to  $120^\circ$ , but because its insertion is eccentric to the transverse axis of joint rotation, the apodeme is pulled distally between  $130$  and  $140^\circ$  (Fig. 1).

### Time-dependent reflex responsiveness

The membrane potentials of some motor neurones in the metathoracic ganglion show a time-dependent and direction-sensitive hysteresis associated with sensory input that is sensitive to the position of the femoro-tibial joint (Siegler, 1981a). In the present experiments it was necessary to control for these effects when delivering phasic stimuli to the femoro-tibial joint.

A posterior flexor tibiae motor neurone was stimulated with a  $20^\circ$  movement of the FCO apodeme ( $30$ – $50$ – $30^\circ$ ) at selected intervals up to 7 min after setting the FCO apodeme at  $30^\circ$  (Fig. 2). Its mean membrane potential did not change by more than  $0.5$  mV during this period. The only difference in its phasic responses to stimulation was that after 5 s it gave a spike (Fig. 2a), whereas to all later stimuli it gave sub-threshold depolarization (Fig. 2b–e). At the other end of the spectrum of such time-dependent effects seen in the leg motor neurones, the slow extensor tibiae motor neurone (SETi) showed a change in its membrane potential that outlasted the tonic change in joint angle by as much as 30 s. To achieve a standardized stimulus regime, therefore, 1 min was allowed to elapse following each tonic change in the position of the joint before phasic stimuli were applied.

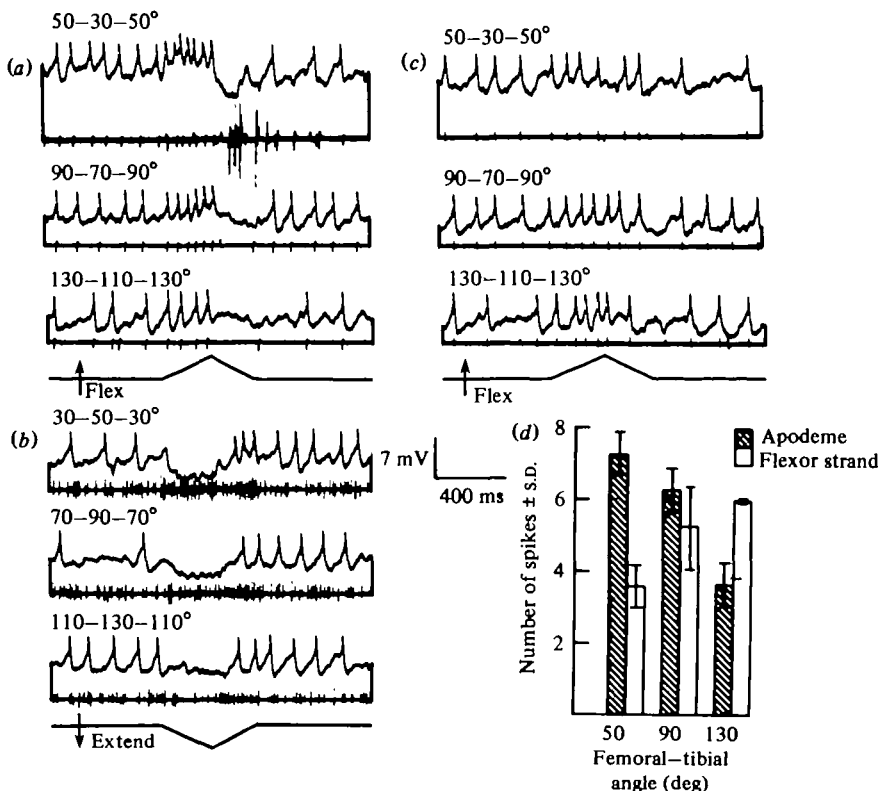


Fig. 3. The effect upon the SETi motor neurone of moving either the apodeme (a, b) or the flexor strand (c) of the FCO by the same amount at three different set angles of the femoro-tibial joint. Intracellular recordings from SETi are on the upper traces, myograms from the extensor tibiae muscle are on the second traces in (a, c). The second trace in (b) is an extracellular *en passant* recording of the sensory discharge from the FCO. The lower trace monitors the movement imposed upon the individual components of the FCO. (a) The maximum excitation produced by the FCO apodeme occurs at 50°. (b) If the movement is now reversed but remains the same amplitude, so that extension precedes flexion, the excitatory effect is reduced. (c) The flexor strand has a weaker excitatory effect than the apodeme, but is more effective at the extended positions. (d) Histograms of the numbers of spikes produced in the SETi by the two parts of the FCO at three different set angles. Mean and standard deviation were derived from ten tests at each angle.

### Responses of extensor tibiae motor neurones

#### The slow extensor tibiae motor neurone (SETi)

If SETi spikes tonically, then a progressive change in the angle of the femoro-tibial joint from flexion to extension, or in equivalent changes in the stretch applied to the FCO apodeme, will inhibit the spikes. At a flexed position, stimulation of the FCO apodeme with continuous triangular movements at frequencies below 5 Hz caused entrainment of the spikes for many minutes without adaptation. The underlying rhythmical fluctuations of the membrane potential sometimes showed a weak adaptation within the first second, but usually remained constant. At an extended position of the femoro-tibial joint, the response to the same stimulus consisted of complex waves of depolarizing and hyperpolarizing potentials (EPSPs and IPSPs) with occasional spikes.

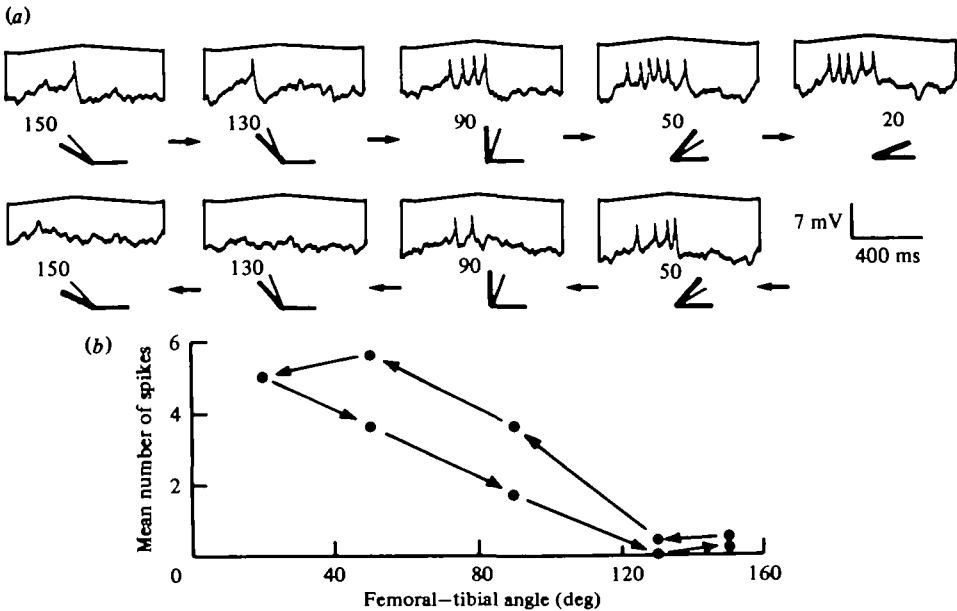


Fig. 4. Hysteresis in the response of the SETi motor neurone. A triangular stimulus of 1.5 Hz and with an amplitude representing a 20° movement of the femoro-tibial joint was applied to the FCO apodeme, at equivalent angular positions from 150° to 30° and back. At each new position of the leg, the constant triangular stimulus was applied only when the position had been attained for 30 s. (a) Intracellular recordings from SETi at various positions and in the sequence in which they occurred. An upward deflection of the top trace indicates a flexion movement. The diagram below each record indicates the equivalent angle through which the FCO apodeme was moved: the thick line represents the starting position of the tibia. (b) Graph of the number of spikes evoked in SETi by the movement at each position. Each point represents the average for ten successive repetitions of the movement at each position.

The response to a single movement of 20° was also dependent upon the set position of the FCO apodeme (Fig. 3). The strongest effect, in terms of the number of spikes evoked, occurred when the apodeme was set at equivalent flexed positions and then moved (e.g. a 50–30–50° movement) (Fig. 3*a–d*). Each repetition of the movement elicited spikes upon flexion and inhibition upon extension. When the stimulus was reversed so that extension preceded flexion the excitation during flexion was now less (compare the 30–50–30° movement in Fig. 3*b* with the 50–30–50° movement in Fig. 3*a*). The flexor strand remained static during these experiments. When it was moved independently of the apodeme it produced maximal effects at the extended set position of the joint (Fig. 3*c, d*).

An hysteresis in the response of the SETi to a movement of the FCO apodeme occurred if the set position was changed sequentially in steps from an extended to a flexed position and then back again (Fig. 4). The number of spikes evoked was greater as the set position was changed towards flexion than it was when changed towards extension (Fig. 4*a, b*).

The response of SETi was also dependent upon the velocity of the movement (Figs. 5, 6). The rate of rise of the depolarization varied with the rate of rise of the triangular stimulus (Fig. 5*a–c*). When records were averaged the greatest amplitude

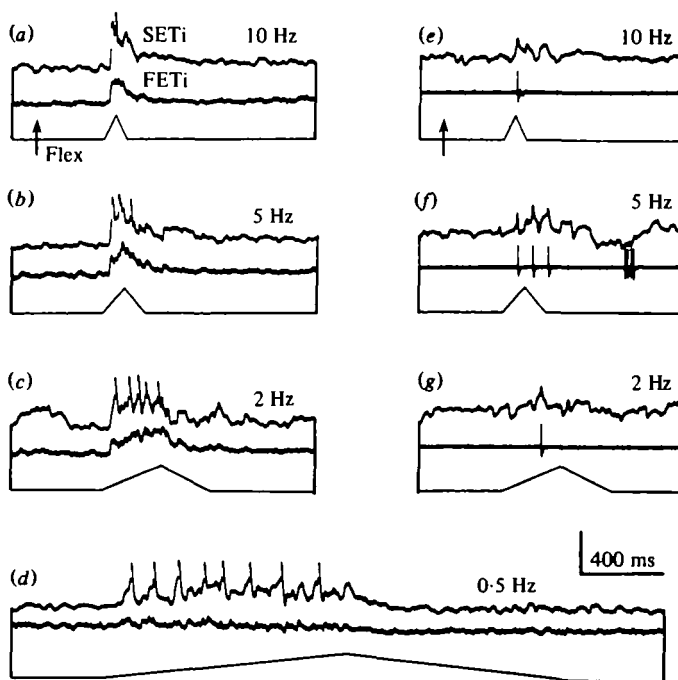


Fig. 5. Effect of velocity of movement of the FCO upon the slow (first traces) and fast (second traces) extensor tibiae motor neurones. (a-d) The FCO apodeme is moved through  $20^\circ$  at 10, 5, 2 and 0.5 Hz from a starting position that corresponds to a femoro-tibial angle of  $50^\circ$ . The higher frequencies evoke a rapid depolarization in both neurones but spikes only in the slow. Lower frequencies evoke sustained spiking in the slow, but have only a small depolarizing effect on the fast. (e-g) The flexor strand is moved through  $20^\circ$  at 10, 5 and 2 Hz (from the same starting point as before), and recordings are made only from the slow motor neurone. The maximal response occurs at 5 Hz with both lower and higher frequencies evoking fewer spikes. The spikes on the second traces are from both the extensor and flexor tibiae muscles. The lower trace throughout represents the stimulus, in which flexion is upwards. Calibration: voltage, (a-d) SETi 7 mV, FETi 4 mV, (e-g) 9 mV.

of depolarization was produced by a  $20^\circ$  stimulus of 5 Hz (Fig. 6a). Stimuli to the apodeme of lower velocity evoked a smaller depolarization and a sustained sequence of spikes (Fig. 5d), whilst similar stimuli to the flexor strand only produced an occasional spike (Figs. 5g, 6a).

#### *The fast extensor tibiae motor neurone (FETi)*

Stimulating the FCO failed to evoke spikes in this motor neurone. The effects on membrane potential were of a similar polarity to those in SETi and showed a similar dependence on the set position of the FCO. The effects of velocity of stimulation were, however, more marked. High velocities evoked a rapid and large depolarization, whereas velocities below 2 Hz produced a progressively smaller change (Fig. 5a-d). In contrast to the peak SETi response to a  $20^\circ$ , 5 Hz stimulus, the FETi gave steadily increasing responses up to a velocity of  $20^\circ$  at 10 Hz (Fig. 6b).



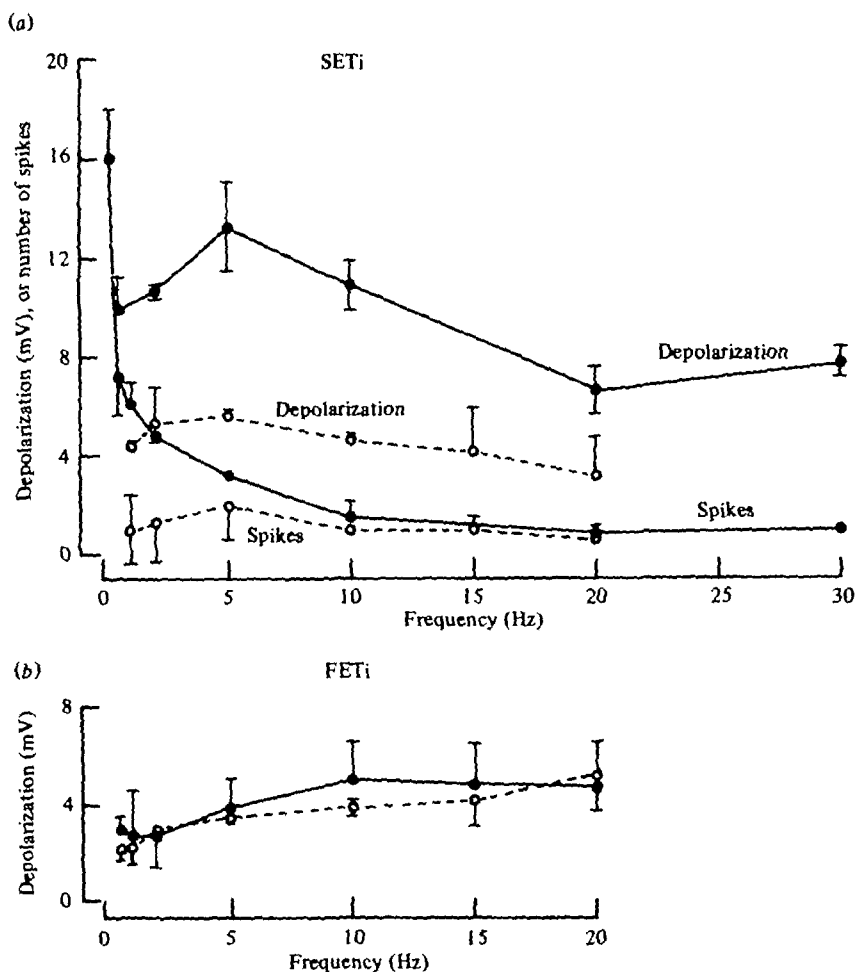


Fig. 6. Graphs of the effects of velocity of FCO apodeme movement on the peak depolarization or number of spikes evoked in the slow (a) and in the fast (b) extensor tibiae motor neurones. The stimulus corresponded to a  $20^\circ$  movement from a starting position of  $50^\circ$ . In the slow motor neurone the depolarization is greatest at the lower frequencies whereas in the fast higher frequencies have a somewhat more pronounced effect.  $\bullet$ — $\bullet$ , Movements of the apodeme;  $\circ$ — $\circ$ , the flexor strand. Means and standard deviations are 2–5 repetitions of the stimulus applied to each of 5 SETi motor neurones in (a) and to 3 FETi motor neurones in (b).

### Responses of flexor tibiae motor neurones

Most flexor motor neurones were depolarized by a movement of the FCO which corresponds to tibial extension. Their responses are thus opposite to those of the extensor motor neurones (Fig. 7). The type of response depended upon the type of flexor motor neurone: fast motor neurones showed a depolarization that rarely evoked spikes, whereas slow motor neurones produced a sequence of spikes often at high frequency. Other flexor motor neurones gave responses intermediate to those of the fast and the slow.

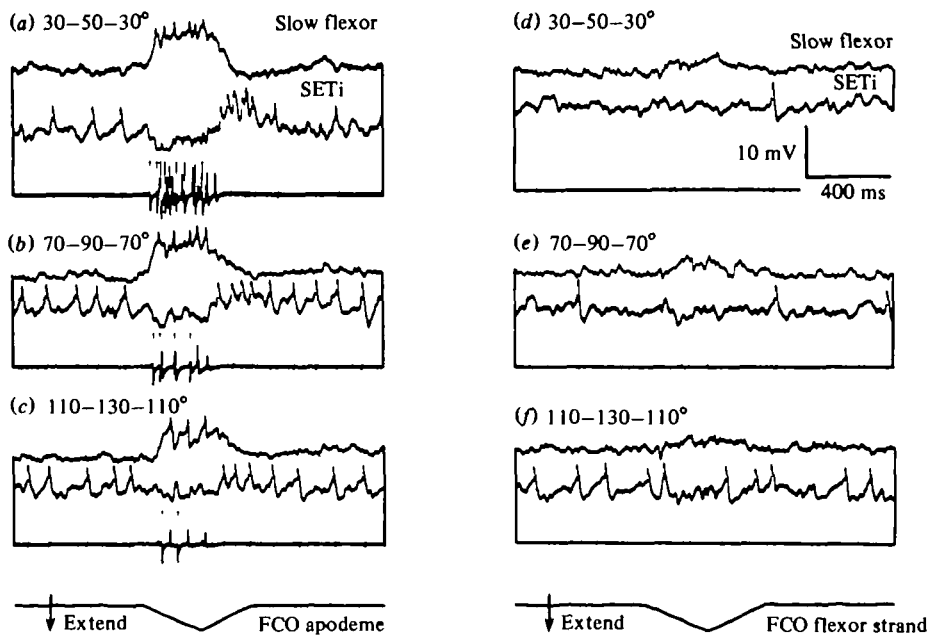


Fig. 7. The effect upon a slow anterior flexor tibiae motor neurone of moving the FCO apodeme (a-c) or flexor strand (d-f) by the same amount at three set positions. Simultaneous recordings were made intracellularly from this flexor motor neurone and from SETi and extracellularly from the flexor tibiae muscle. (a-c) Extension of the apodeme evokes spikes in the flexor and inhibition in the SETi. Both effects are greatest for the 30-50-30° movement. (d-f) Movement of the flexor strand has a much weaker effect upon both motor neurones and does not evoke spikes in either.

### *Effect of set position*

As for the extensor motor neurones, the responses to a single movement of the FCO apodeme were dependent upon the set position. The strongest response was evoked at the more flexed positions (Fig. 7a, b), though spikes were still evoked in some motor neurones at extended positions (Fig. 7c). If the stimuli were applied repetitively, the dependence upon set position became more striking (Fig. 8). At a flexed set position, each extension movement evoked a large depolarization in a fast flexor motor neurone and spikes in other flexor motor neurones which alternated with those in the SETi (Fig. 8a). If the set position of the FCO apodeme was 90°, the rhythmical depolarization of the fast flexor was reduced as was the number and frequency of spikes in the other flexors and in SETi (Fig. 8b). With a set position of 130°, the depolarization in the fast flexor was further reduced, no spikes were evoked in other flexors, but the frequency of spikes in SETi was still modulated rhythmically (Fig. 8c).

### *Effectiveness of the two FCO components*

Movement of the FCO apodeme was a more effective stimulus than movement of the flexor strand. Comparison of the effects of the apodeme and the flexor strand upon the same flexor motor neurone in the same locust showed that the apodeme could elicit spikes at all set positions (Fig. 7a-c), but that the flexor strand could elicit

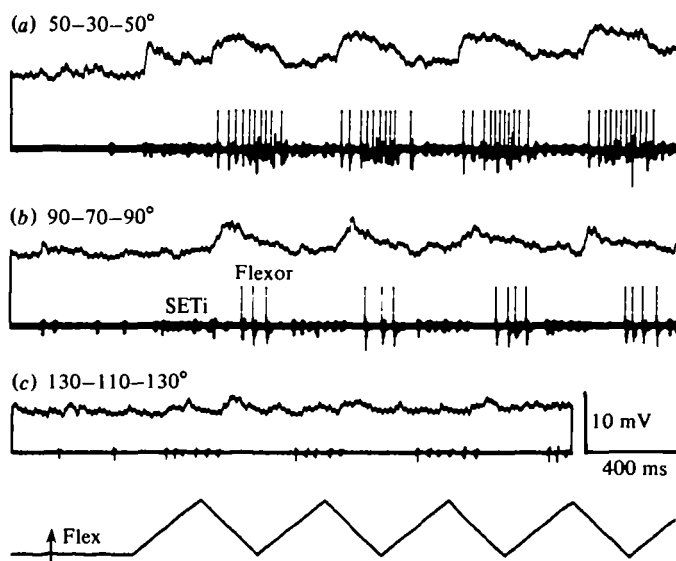


Fig. 8. Responses of a fast flexor tibiae motor neurone to movements of the FCO. (a-c) The FCO apodeme is moved repetitively at 2 Hz with an amplitude equal to  $20^\circ$  at three different set positions. The effect is greatest both upon the intracellularly recorded subthreshold response of a posterior flexor motor neurone and upon the spikes of flexor and the slow extensor motor neurones recorded extracellularly in the femur when the set position is  $50^\circ$ . Note the depolarization of the flexor motor neurone in (a) that occurs on the initial flexion movement.

only a small depolarization at each (Fig. 7d-f). There was little difference in the effectiveness of the flexor strand at the various set positions.

These effects of the two components of the FCO and of the set position occurred in most of the flexor motor neurones examined. In all but 3 of 27 flexor motor neurones, the effect of the FCO apodeme was maximal at the most flexed set position. By contrast, the effectiveness of the flexor strand was not dependent on the set position in any of the 27 flexor tibiae motor neurones examined.

### Effect of velocity

The response of flexor motor neurones was dependent also upon the velocity of the movement applied to either component of the FCO. Slow motor neurones responded to fast movements of the FCO apodeme with a rapid initial depolarization whose rate of rise varied with that of the stimulus (Fig. 9a). The slower and longer the stimulus, the greater was the amplitude of the depolarization and the longer was the depolarization sustained. Hence the slower and longer stimuli often evoked a sustained sequence of spikes (Fig. 9a). Responses to movements of the flexor strand showed a clear dependence upon velocity although the amplitude of the depolarization was less. In fast motor neurones, the effect of velocity was the converse of that in the slow motor neurones; fast stimuli evoked the greatest depolarizations (Fig. 10). Stimulation of the FCO apodeme with a  $20^\circ$ , 20 Hz triangular movement produced a more rapid depolarization than that in slow motor neurones, and usually evoked spikes (Fig. 10a). Reducing the velocity of the stimulus reduced the amplitude of the

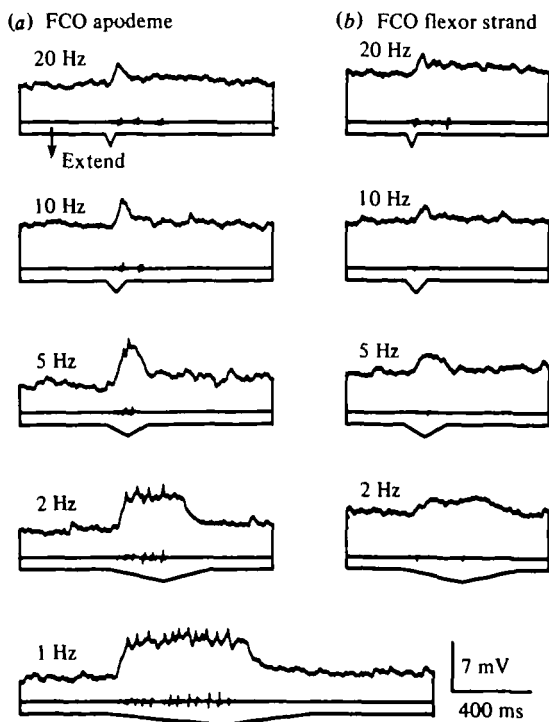


Fig. 9. Effect of the velocity of the movement of either the FCO apodeme (a) or the flexor strand (b) on a slow flexor tibiae motor neurone. The myogram from the flexor tibiae muscle is on the second trace. The set position was  $30^\circ$  and the stimulus was an extension movement of  $20^\circ$  at the frequencies indicated. Extension is indicated by a downward deflexion of the lower traces. (a) All velocities of movement to the apodeme elicit a substantial depolarization, but the response to the lower ones is more sustained and results in a sequence of spikes. (b) The flexor strand elicits a smaller depolarization at all velocities.

depolarization although it remained more rapid in its rise than that seen in a slow motor neurone. Slow stimuli evoked responses that declined quickly at first and then more slowly. Occasionally there was a depolarization at the end of a stimulus. Fast motor neurones are therefore unable to sustain the full amplitude of depolarization throughout a slow stimulus. Responses evoked by movements of the flexor strand were similar to those evoked by the apodeme (Fig. 10b).

The most effective velocity of stimulus, defined as the one which evoked the largest amplitude of depolarization in a motor neurone, was different for the various flexors (Fig. 11a, b). In addition to the slow and the fast described above were other flexor motor neurones whose maximal response was elicited by stimuli of different velocities.

#### *Effect of repetitive stimuli*

Flexor motor neurones also differed in their rates of adaptation to repetitive movements of the FCO apodeme or flexor strand (Fig. 12a, b). Three basic types of response could be recognized. First, motor neurones which were depolarized during the first few seconds of stimulation but which then adapted rapidly to a response that was barely detectable from background (Fig. 12a, open circles). Secondly, motor neurones

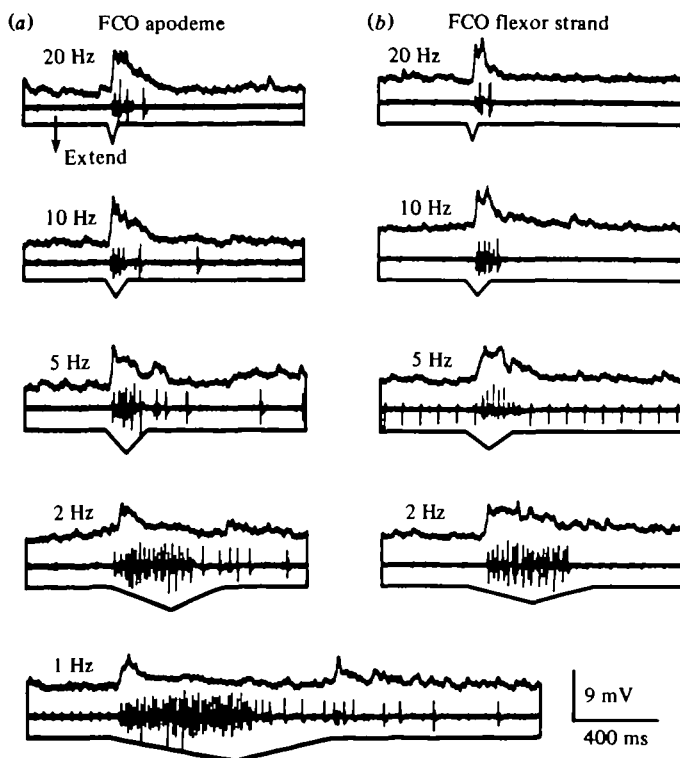


Fig. 10. Effect of velocity of FCO movement upon a fast flexor tibiae motor neurone. The experimental details are the same as in Fig. 9. The myogram on the middle traces monitors spikes in flexor and extensor tibiae motor neurones. (a) The FCO apodeme elicits a depolarization at all frequencies, but at the lower ones the response in the motor neurone is not sustained. (b) The flexor strand also elicits a similar level of depolarization that is more sustained at the lower frequencies.

whose response after 10 s of stimulation was little different from that at the start (Fig. 12*a, b*, triangles). There was some summation of the depolarizing waves so that they were superimposed upon a tonic shift in the membrane potential. Thirdly, motor neurones whose initial response was a large depolarization often with spikes (Fig. 12*a, b*, closed circles). This response declined within 1–3 s of stimulation, but the subsequent responses were still prominent fluctuations of the membrane potential at each cycle of the stimulus. In general fast motor neurones fall into the first category, intermediate ones into the second, and slow ones into the third.

#### *Effect of reversing the direction of movement*

When, at the midpoint of a triangular waveform, the direction of stimulation applied to the FCO changed from extension, which was usually excitatory, to flexion, which was usually inhibitory, the response of a flexor motor neurone similarly reversed in polarity (e.g. Figs. 9, 10). Often, however, the reversal of the response was delayed so that an excitation persisted past the midpoint of a stimulus (Fig. 13). These delays can be two orders of magnitude greater than the conduction times of the sensory and motor impulses that are involved in the reflex pathway. In Fig. 13(*a*), a single stimulus of

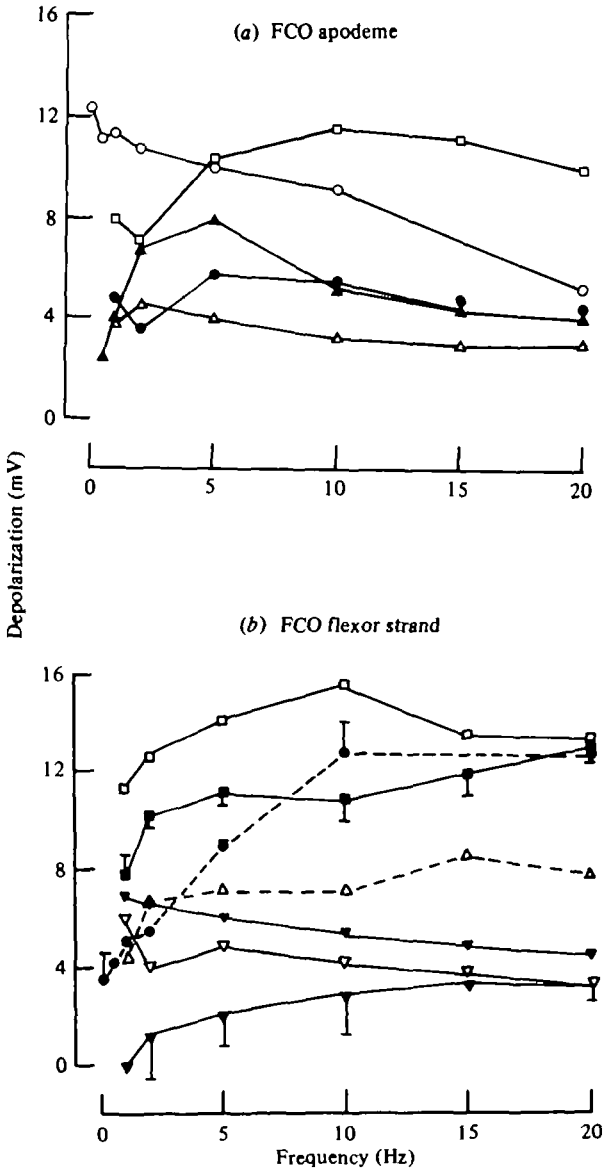


Fig. 11. Graphs to show the effect upon flexor motor neurones of altering the velocity of extension movement of the FCO apodeme (a) or flexor strand (b). The stimuli used corresponded to a movement of the femoro-tibial joint of  $30^{\circ}$ - $50^{\circ}$ - $30^{\circ}$ . The mean depolarization for 2-5 trials of a  $20^{\circ}$  movement at each velocity is shown for five different flexor motor neurones in (a), and for seven in (b).

10 Hz applied to the FCO apodeme evoked a depolarization in a lateral fast flexor motor neurone. Repolarization started towards the end of the flexion phase of the movement and ended some 100 ms after the movement (Fig. 13a). Similarly during repetitive movements the repolarization was delayed at each cycle (Fig. 13b). The delayed repolarization means that spikes are often evoked during the flexion phase of the movement (Fig. 13c). That these delays are a common effect in flexor motor

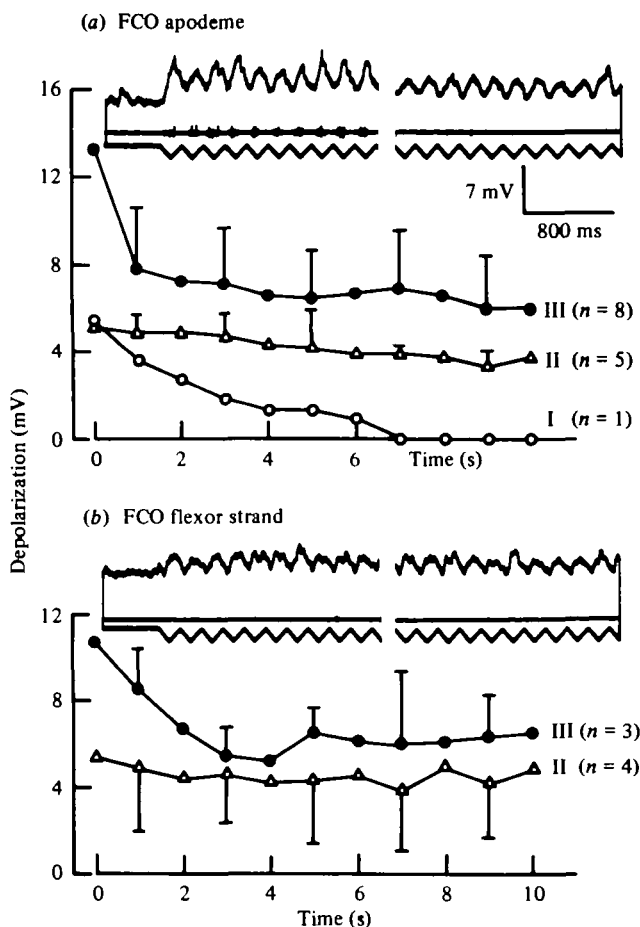


Fig. 12. Adaptation of flexor tibiae motor neurones to repetitive movements of the FCO. The movement was equal to a  $20^\circ$  excursion of the femoro-tibial joint repeated at a frequency of 5 Hz for 10 s. (a) The graph shows mean and standard deviation adaptation rates for three types of response to stimulation of the FCO apodeme. (b) The adaptation rates of the same motor neurones as in (a) represented by open triangles and by closed circles, to movements of the flexor strand. The recordings in (a) and (b) are from a motor neurone with the characteristics of those plotted with the open triangles.

neurones is apparent when their responses are plotted against the phase of a single stimulus applied to the FCO apodeme (Fig. 13e). Extensor motor neurones also behaved in a similar way (Fig. 13c, d). In response to a movement that evoked a persistence of spiking in a flexor motor neurone into the flexion phase, the SETi was depolarized only at the very end of the movement (Fig. 13c). During repetitive stimulation, SETi was depolarized in both the flexion and extension phases of the movements (Fig. 13d).

Sometimes, either a flexor or an extensor tibiae motor neurone was actively excited during both phases of the stimulus (Fig. 14). An excitatory response to a movement that was normally inhibitory could occur transitorily in a repeated sequence of movements. For example, in a sequence of triangular stimuli, a fast flexor was depolarized

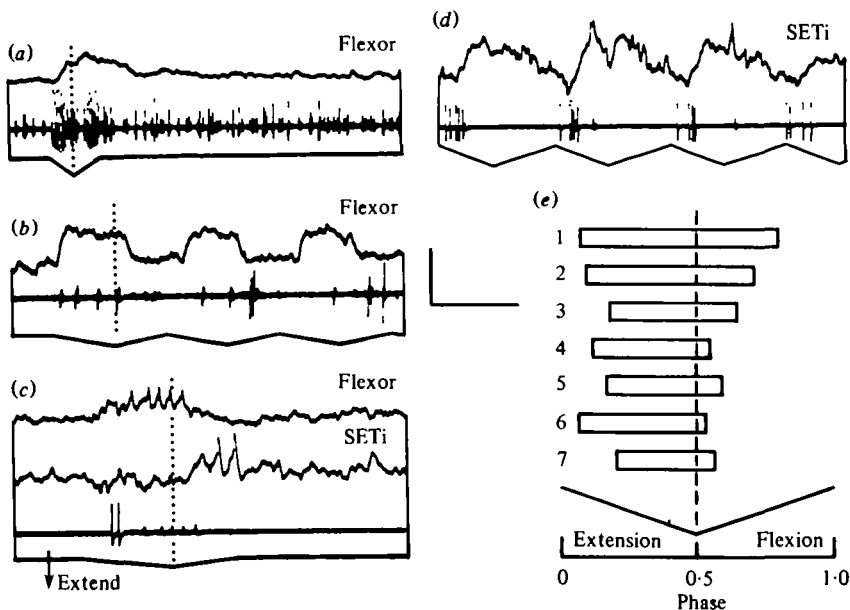


Fig. 13. Delayed responses of motor neurones. (a) A lateral fast flexor is repolarized only when movement of the FCO apodeme ( $30-50-30^\circ$  at 10 Hz) has been completed. (b) During three cycles of movement ( $30-50-30^\circ$  at 2 Hz) of the FCO apodeme there is delayed repolarization of a lateral flexor. (c) The anterior slow flexor (upper trace) continues to spike during the flexion phase of the movement ( $110-130-110^\circ$  at 1.5 Hz). The SETi (second trace) is depolarized only towards the end of flexion. (d) The SETi is depolarized during both the extension and flexion phases of the FCO apodeme movement ( $50-30-50^\circ$  at 2 Hz). (e) Mean ( $n = 6$ ) phase of the period of a burst of spikes in seven flexor tibiae motor neurones in response to a movement of the FCO apodeme representing a change in the angle of the joint from  $30-50-30^\circ$ . The second trace in (b, d) and the third trace in (c) are myograms from the flexor tibiae muscle. The second trace in (a) is a recording from the FCO nerve. Calibration: voltage, (a-c) 9 mV, (d) 4.5 mV; time, (a) 200 ms, (b-d) 400 ms.

by the flexion movement in the first cycle in addition to the normal depolarization by extension (Fig. 8a). On other occasions the depolarization to both directions of movement persisted through many applications of the same stimulus, lasting several minutes. A single triangular movement evoked a depolarization and spikes in a slow flexor upon both the flexion and extension phases of the movement (Fig. 14a). A similar effect occurred in a fast flexor regardless of which direction of movement was implemented first (Fig. 14b, c). The excitation on each phase of the movement was additive in a flexor motor neurone as shown by presenting the stimulus twice, but with the phases of movement reversed upon the second occasion (Fig. 14d, e).

#### *Responses of tarsal motor neurones*

When the tibia is forcibly extended the tarsus is depressed and when the tibia is forcibly flexed the tarsus is levated (Burrows & Horridge, 1974). Similarly, tarsal motor neurones show a strong compensatory reflex when the FCO apodeme is moved. The compensatory movements of the tarsus involve the excitation of motor neurones to one tarsal muscle and the inhibition of those to the other. A movement of the FCO apodeme corresponding to extension of the tibia, inhibited the normal background



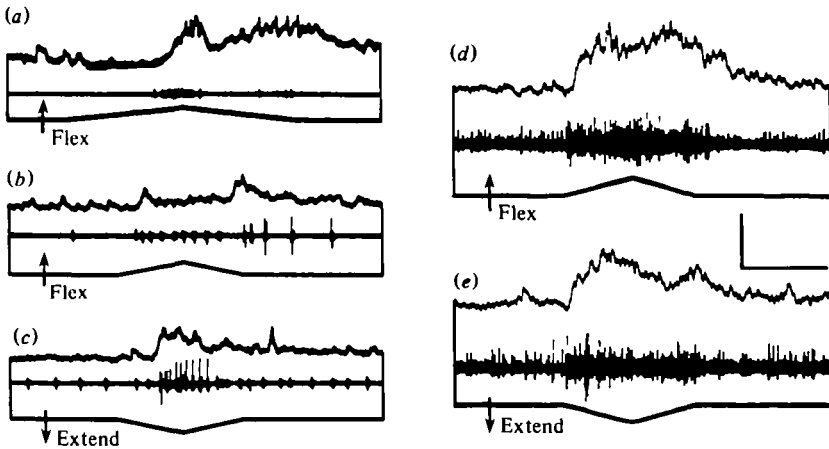


Fig. 14. A motor neurone may be excited by movements of the FCO that correspond to both flexion and extension. (a) A slow flexor is depolarized at the end of a flexion movement of the FCO apodeme as well as during extension (movement was  $50-30-50^\circ$ ). (b) A lateral flexor is depolarized twice during a  $50-30-50^\circ$  movement of the flexor strand. (c) During the reciprocal movement it is again depolarized. (d) A posterior flexor is also depolarized during both phases of a  $50-30-50^\circ$  movement of the FCO apodeme. (e) The reciprocal movement is excitatory during both phases. The second trace in (a-c) is a myogram from the flexor and extensor tibiae muscles, and in (d, e) is a recording from the FCO nerve. Calibration: voltage, (a) 7 mV, (b, c) 9 mV, (d, e) 5 mV; time (a) 800 ms, (b-e) 400 ms.

spiking in the levator tarsi motor neurone (Fig. 15a-d). The inhibition is brought about by large IPSPs, one class of which is evoked by a local spiking interneurone (Burrows & Siegler, 1982). The effect of the FCO afferents is thus not mediated by a direct connexion with the motor neurone. The effectiveness of the FCO was markedly dependent upon its set position. At a flexed position of  $30^\circ$ , the inhibition was large, and was followed by excitation that led to an increase in the frequency of spikes during the flexion phase of the movement (Fig. 15a). As the set position was changed gradually towards extension, the inhibition was reduced and the excitation abolished (Fig. 15b-d). The response of the motor neurone was different if the FCO was moved through the same angle but with flexion now preceding extension (Fig. 15e-g). At a flexed position, the initial flexion evoked an increase in the frequency of spikes followed by inhibition during extension (Fig. 15e). At a mid position, there was no excitation during flexion but a slight inhibition remained during extension, causing the frequency of spikes to fall transiently (Fig. 15f). At an extended position, however, there was no effect when the FCO was moved (Fig. 15g). This was in contrast to the still noticeable effect at this set position for a movement in the reverse sequence.

The effects upon tarsal depressor motor neurones were opposite to those on the levator; movement of the FCO corresponding to extension excited, whilst one corresponding to flexion inhibited. Both the FCO apodeme and the flexor strand elicited qualitatively similar effects that were dependent upon the set position.

#### *Changes in responsiveness of the motor neurones*

In addition to phase-related changes in the responses of motor neurones to movements of the FCO, there were more general changes in responsiveness that became

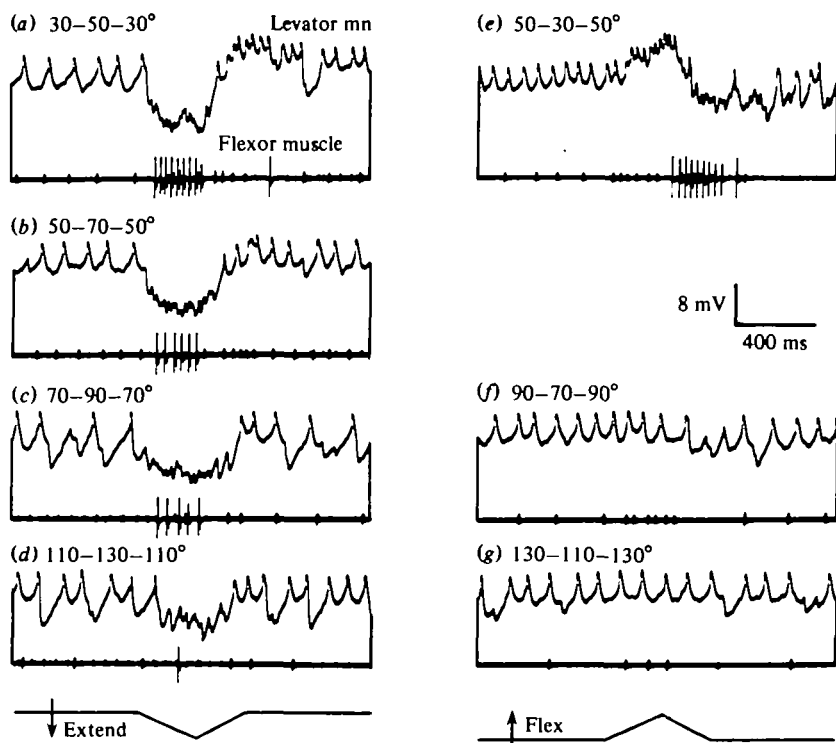


Fig. 15. Effects on the levator tarsi motor neurone of moving the apodeme of the FCO. The stimulus to the apodeme corresponds to a movement of the femoro-tibial of  $20^\circ$ , at different set positions. (a-d) When an extension movement precedes a flexion there is an inhibition of the spikes. The inhibition is greatest at the more flexed set positions. (e-g) When flexion precedes extension the inhibition is reduced and is apparent only at the most flexed positions. The upper traces are intracellular recordings from the levator motor neurone, the second traces myograms from the flexor tibiae muscle.

apparent during repetitive stimulation (Fig. 16). These changes could occur without obvious correlation with motor activity in other limbs or changed sensory stimuli of any modality (Fig. 16*b, d*). At other times they could be correlated with elicited motor acts (Fig. 16*a*). For example, when the contralateral hind leg was touched, the SETi was inhibited and the preceding excitatory response to the FCO stimulation could no longer be seen (Fig. 16*a*). The response of a flexor motor neurone that was depolarized upon each extension, was modified during voluntary movements of the hind leg (Fig. 16*c*). On other occasions the response of a flexor motor neurone gradually increased in amplitude to a repetitive movement before declining to its previous level (Fig. 16*b*). The change in responsiveness in one motor neurone was not necessarily reflected in a similar change in another motor neurone to the same leg. For example, a simultaneous recording from a flexor and the fast extensor motor neurone showed two transient changes in the amplitude of the response of the former whilst that of the latter remained at a consistent level (Fig. 16*d*). The responses elicited by the FCO are thus to be seen as basic recognizable patterns, but upon which changes can be superimposed due to the activity of other neurones in the central nervous system. Therefore the reflex responses of the motor neurones are not invariant.

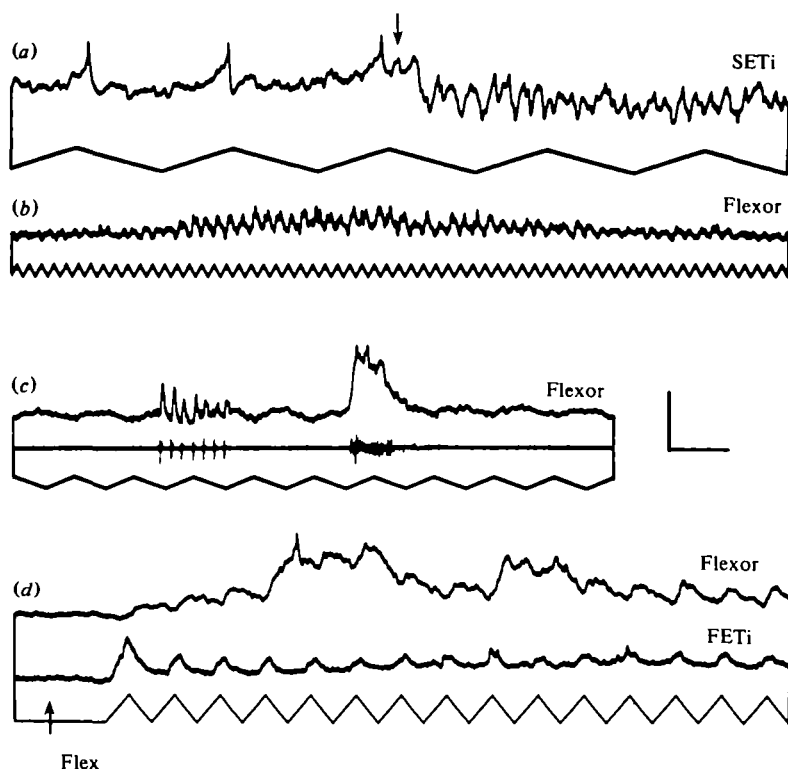


Fig. 16. Changes in the responsiveness of motor neurones to repeated movements of the FCO. (a) The SETi spikes once at each flexion movement of the FCO apodeme, but when the contralateral hind leg is touched (arrow) this response is masked by large hyperpolarizing potentials. (b) The response of a fast flexor spontaneously increases and then wanes to a continuous 10 Hz movement of the FCO apodeme. (c) Spontaneous movements of the leg occur during a continuous 2 Hz movement of the FCO apodeme. (d) A lateral flexor (upper trace) shows two increases in its response to a repetitive movement of the FCO apodeme but such changes are not seen in the FETi recorded at the same time. Calibration: voltage, (a-c) 7 mV, (d) 5 mV; time, (a, c, d) 400 ms, (b) 800 ms.

## DISCUSSION

In earlier investigations of the physiology of the femoral chordotonal organ of locusts (Usherwood *et al.* 1968; Burns, 1974), the flexor tibiae muscle was removed when the sense organ was prepared for recording. This would probably have destroyed one of the distal attachments of the organ, the flexor strand. In the present experiments the two distal attachments, the flexor strand and the apodeme, remained intact and were stimulated separately. Movement of the femoro-tibial joint causes reciprocal movement of these two components and hence it might be expected that their reflex effects would be different. This expectation has been confirmed for the two muscles that move the tibia, and for the two tarsal muscles that are involved in an inter-joint compensatory reflex. In each instance the strongest effects occurred during maximum stretching of either of the two components. For example, the flexor strand was usually most effective at the extended positions of the joint, whereas the apodeme was most effective at flexed positions. Both components produced maximum

responses to an increase or decrease in tension when held in their most stretched state. This, plus the observation that the motor neurone responses to movement of the two FCO components often differed qualitatively, make it unlikely that movement of the flexor strand provides parallel mechanical stimulation to the same population of sensory neurones that are activated by the apodeme.

The femoral chordotonal organ mediates many of the reflex effects that have been previously described to result from movement of the femoro-tibial joint. Stimulation of the FCO in ways that correspond to tibial extension excites the flexor tibiae and depressor tarsi motor neurones, whilst stimulation corresponding to tibial flexion excites extensor tibiae and levator tarsi motor neurones. At low velocities of movement, the slow motor neurones contribute most to the reflex efference to the muscles of the leg. At high velocities of movement, the responses of the slow motor neurones are reduced whilst those of the fast motor neurones are enhanced.

Excitation of flexor and the slow extensor tibiae motor neurones sometimes occurred during both flexion and extension phases of the stimulus. The effect on the flexor motor neurones is, for example, to increase their excitability during the flexion phase of the movement. These unexpected effects serve to emphasize the complexity of the control mediated by the femoral chordotonal organ, and strongly indicate that it plays a role in locomotion as well as in posture.

Passive movements of the joints in arthropods often elicit responses in muscles that are out of phase with resistance reflexes (Clarac & Dando, 1973; Di Caprio & Clarac, 1981; Field & Rind, 1981). These include the phase overlap of the reflex in the flexor motor neurones reported here, and the phase reversal of an apparent resistance reflex into an assistance reflex in insects and crustaceans (Bässler, 1976; Vedel, 1980; Di Caprio & Clarac, 1981). These experiments have usually not permitted the legs to contact the substrate so that it is not certain that the effects would be seen in an animal that was standing or allowed to walk normally. Receptors that signal contact of the legs with the substrate are well known (Runion & Usherwood, 1968; Kendall, 1970; Shelton & Laverack, 1968) and their effects may well interact with those mediated by other leg proprioceptors such as the femoral chordotonal organ.

In crustaceans (Clarac, Vedel & Bush, 1978) and insects (Field & Rind, 1981) chordotonal organs mediate reflexes that affect several joints of a particular limb. These interjoint reflexes often appear together with resistance reflexes when a joint is moved passively: for example, in the locust, the levator tarsi motor neurone is excited when the tibia is flexed. Often these effects are synergistic in that they would augment the normal walking movements of the animal (see also Burrows & Horridge, 1974; Ayers & Davis, 1977). A major task that must now be undertaken is to study walking insects in which it would be possible to test for the presence of synergistic reflexes and thereby assess their role in locomotion.

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