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THE ROLE OF FAST EXTENSOR MOTOR ACTIVITY IN THE LOCUST KICK RECONSIDERED

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Summary

Fast extensor tibiae (FETi) activity has been implicated as a crucial element underlying the locust kick motor programme with regard to four circuits. (i) A positive feedback reflex from extensor tibiae (ETi) muscle tension helps maintain FETi spiking during co-contraction. (ii) A central connection from FETi to flexor tibiae (FlTi) motor neurones helps initiate FlTi spiking at the start of co-contraction. (iii) Reflex feedback from ETi tension to FlTi motor neurones helps maintain the latter spiking during co-contraction after the central connection has decremented. (iv) A proprioceptive gate controlled by ETi tension ensures that FlTi trigger activity does not occur until sufficient ETi tension has developed to allow an effective kick.

The hypotheses concerning these circuits have been tested in two ways. First, FETi was phasically inhibited for varying periods during co-contraction, abolishing its spikes and hence its central output, and reducing ETi tension. Second, the nerve containing the FETi axon was cut in the femur, thus partially denervating the ETi muscle, and reducing its tension without directly affecting FETi activity. In both cases, kicks were analysed to see whether the motor programme changed in accordance with the circuit model. The overall conclusion is that the model is not correct, since considerable experimentally induced changes in FETi activity and ETi tension had no obvious effects on the motor programme. The circuits may play a supplementary role in generating the programme, but they are not crucial to it.

Introduction

The locust jumps and kicks by the rapid extension of its metathoracic tibiae. This powerful movement requires a three-stage motor programme for each leg

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(Godden, 1975; Heitler & Burrows, 1977*a*). First, the locust flexes the metathoracic tibia by activating flexor tibiae (FlTi) motor neurones. This is the cocking phase (Pearson & Robertson, 1981). Second, the extensor tibiae (ETi) muscle contracts, driven by the fast extensor tibiae (FETi) motor neurone. While this happens the tibia is maintained flexed by concurrent FlTi motor activity. This is the co-contraction phase. Finally, the FlTi muscle relaxes suddenly, allowing the tibia to extend. This is the trigger phase. It results from the simultaneous inhibition of FlTi excitatory motor neurones and excitation of FlTi inhibitory motor neurones. An interneurone called M has been identified which inhibits FlTi excitatory motor neurones and which gives a burst of spikes at the end of co-contraction with precisely the right timing to underlie the trigger activity (Pearson, Heitler & Steeves, 1980; Gynther & Pearson, 1986).

FETi motor activity has been regarded as a crucial element underlying this programme. Four circuit features have been implicated (Heitler & Burrows, 1977*b*; Pearson, 1983).

(1) An excitatory reflex occurs onto FETi if it spikes when the tibia is held flexed (Hoyle & Burrows, 1973). It has been suggested that this positive feedback leads to an avalanche of excitation, and helps to produce the FETi burst during co-contraction (Hoyle & Burrows, 1973).

(2) A strong central excitatory connection exists between FETi and the FlTi motor neurones (Hoyle & Burrows, 1973). This has been suggested to be involved in the initiation of FlTi activity at the start of co-contraction (Hoyle & Burrows, 1973), although recent observations of variations in timing indicate that it is not essential (Gynther & Pearson, 1986).

(3) The central FETi–FlTi connection is augmented by reflex excitation onto the FlTi motor neurones if FETi spikes with the tibia flexed (Heitler & Burrows, 1977*b*). The central connection decrements rapidly with repetition, and so it has been suggested that this reflex helps to maintain FlTi activity during the later stages of co-contraction (Heitler & Burrows, 1977*b*).

(4) The M interneurone is depolarized by sensory input resulting from FETi activity. It was originally proposed that M is slowly depolarized during co-contraction as a result of sensory feedback from ETi tension, and that the trigger activity was initiated when it finally crossed threshold (Steeves & Pearson, 1982). The attractiveness of this scheme is that a kick will only be released when sufficient ETi tension has been obtained to render it behaviourally effective. However, it later became apparent that M is in fact *hyperpolarized* during co-contraction, and that its terminal burst results from excitatory input from some other source (Gynther & Pearson, 1986). It still remains possible, however, that the unidentified trigger burst generator is itself subject to a tension-dependent proprioceptive gate of the type originally proposed for M.

The circuits described above certainly exist, but the functions ascribed to them are hypothetical. The experiments described in this paper have been designed to test whether they really are important in controlling the basic characteristics of the

motor programme. Two experimental approaches were used. The first was to insert a microelectrode into the cell body of the FETi neurone and to inject a pulse of hyperpolarizing current during the kick motor programme. This abolished FETi spikes for the duration of the pulse, with a consequent reduction both in ETi muscle tension and in the central excitation of the FlTi motor neurones. The second approach was to cut the ETi motor nerve within the femur so as to isolate between one- and two-thirds of the ETi muscle fibres from their innervation and so reduce ETi tension.

The circuit model predicts several consequences from these procedures. The overall reduction in ETi tension should reduce the positive feedback onto the motor neurones during co-contraction, thereby reducing their spike frequency. The rate of excitation of the trigger system would also be reduced, and consequently the trigger activation should be delayed. Thus, according to the model, the outcome should be a less intense but prolonged bout of co-contraction. The phasic hyperpolarization of FETi would also be expected to have effects dependent on its timing. A hyperpolarizing pulse at the beginning of co-contraction might abort the kick by breaking the positive feedback onto FETi mediated by tension produced by the early spikes. It would also prevent the central excitation of the FlTi motor neurones, and thus weaken their activity early in co-contraction. Later hyperpolarization might terminate or greatly weaken the FETi burst. This should reduce both the central and peripheral excitation of the FlTi motor neurones, and reduce the excitation of the trigger system, possibly leading to a kick without proper trigger activity.

Materials and methods

Adult locusts (*Schistocerca gregaria*) of either sex were used. The locust was restrained on its back in Plasticine, and the ventral cuticle dissected to expose the meso- and metathoracic ganglia. All peripheral nerves from these ganglia were cut other than the nerve 5 innervating the ipsilateral leg. This abolished any contralateral sensory influences, which was important since, when the innervation of the contralateral leg was intact, it usually kicked more-or-less synchronously with the ipsilateral leg. The procedure also abolished the effects of the ipsilateral slow extensor and common inhibitor motor neurones, and reduced any variability introduced by other sensory input. The ganglion was washed with saline (Hoyle & Burrows, 1973).

Kicks were usually induced by gently tickling the abdomen with a brush. The kick motor programme is a very definite and specific behaviour which may or may not occur in response to this stimulus: it is not an inevitable reflex consequence. In other words, the motor programme is not directly generated by the stimulus. Often several kicks occurred in succession, only the first of which was directly stimulated. No difference was observed in any of our results between kicks resulting from an external stimulus, and spontaneous kicks.

FETi current inhibition

A procedure similar to that developed by Hoyle & Burrows (1973) was used to place microelectrodes in the cell bodies of the FETi and various FlTi motor neurones. The FETi electrode was low-resistance (5–10 MΩ) to facilitate the passage of large currents. The bath potential was stabilized using a virtual-ground current monitor with separate voltage-recording and current-passing indifferent electrodes (Purves, 1981). Negative current pulses were injected into FETi, using a pulse generator that was triggered with varying delay off spikes recorded from the ETi myogram. In this way FETi spikes were blocked at various points in the co-contraction, and for varying durations. Occasionally a single FETi spike occurred before the onset of the co-contraction burst, and this could be used to trigger an inhibitory pulse before the start of co-contraction proper. The inherent variability of the kick programme meant that the timing of current inhibition relative to the kick programme could not be precisely controlled.

Nerve section

A window was cut in the ventral femoral cuticle located between the first two FlTi muscle insertion sites distal to Brunner's organ, and between the two stiffening longitudinal ridges that run along the ventral surface of the femur (Fig. 1). Fine glass hooks were used to 'fish' for the ETi nerve between the FlTi muscle bundles, and bring it to the surface of the femur where it could be cut. A successful cut in this region usually sectioned the nerve just proximal to a major fork in the distal femur, as determined visually by dissecting the femur following the experiment. Great care had to be taken not to damage the FlTi muscle or the nerves 5B1 and 5B2, which run close to the ETi nerve. Damage to these structures almost invariably resulted in a subsequent failure to induce the locust to kick. The

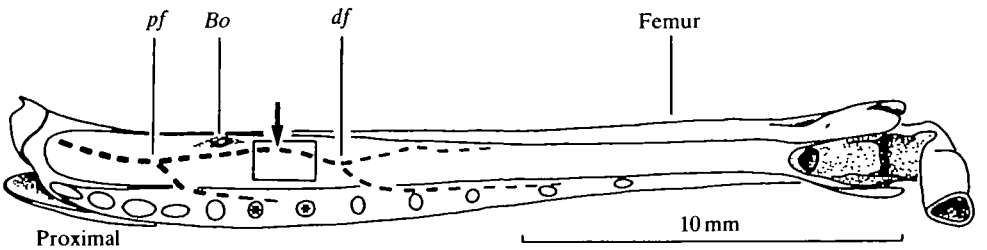

Fig. 1. A ventral view of the femur of the right hindleg of a locust. The dotted line indicates the course of the nerve innervating the ETi muscle. The rectangle shows the location of the window cut in the femoral cuticle, and the arrow the point where the ETi nerve was usually cut. The two FlTi muscle insertion points marked with stars indicate the markers used in deciding the location of the window. *pf*, proximal fork in ETi nerve; *Bo*, Brunner's organ; *df*, distal fork in ETi nerve.

cuticle window had to be kept as small as possible; otherwise the femoral cuticle tended to collapse as soon as the locust tried to kick.

Muscle tension

To measure relative ETi muscle tension, a force transducer was applied to the end of the tibia held at right angles to the femur. The FETi axon was stimulated using hook electrodes placed on nerve 5 within the thorax, and ETi activation was confirmed by myogram recordings from the muscle. Two types of stimuli were applied.

In the current-inhibition experiments an exact duplicate of the FETi spike pattern recorded in the experiment was produced by a computer-controlled stimulator, and used to stimulate the FETi axon in a different preparation. Control experiments showed that when the same pattern was used to stimulate several different preparations, there was some variation in the absolute levels of tension developed, but the shape and relative amplitude of the tension was constant. Thus it is legitimate to determine the effects on relative tension of changes in FETi spike pattern recorded from one single preparation, using another single preparation as a bioassay. It is not possible, however, to compare absolute levels of tension from different preparations with this technique. In tension-measuring experiments each stimulus pattern was repeated three times, and yielded virtually identical tension. The longest experiment involved a sequence of 30 stimulated kicks, and after the last stimulation the initial patterns were repeated. The measured tension had declined by 6–8 % for the same stimulus pattern, indicating that the preparation had suffered little fatigue in the course of the experiment.

In the nerve-cut experiments, single-pulse and simple pulse-train stimuli were used to determine the relative drop in ETi tension following nerve section. Tension measurements were made immediately before and immediately after the cut. The transducer had to be removed during the operation and then replaced, but control experiments showed that with careful positioning highly consistent and reproducible tension measurements could be obtained.

The only axon innervating the ETi muscle which could have been stimulated in these procedures other than FETi was that of the dorsal unpaired median neurone (DUMETi; Hoyle, Dagan, Moberly & Colquhoun, 1974). In preliminary control experiments the contralateral nerve 5 was left intact, and recordings made from it were used to monitor DUMETi activity. The DUMETi threshold was always considerably above that of FETi, and there was in any case no detectable difference in ETi tension resulting from FETi plus DUMETi stimulation compared with FETi stimulation alone. FlTi axons also run in nerve 5, but again have higher thresholds than FETi. In the nerve-cut experiments, nerve 5 was left attached to the ganglion, and FlTi units were sometimes recruited by the central connection. However, the ETi muscle is so much stronger than the FlTi muscle that FlTi activation had little or no effect on the extension force measured at the end of the tibia.

Statistical analysis

Kick performance was analysed by quantifying various rather simple aspects of the motor programme such as duration of co-contraction, number of spikes etc. These are displayed graphically and analysed statistically for differences between control and experimental kicks. Because in many cases the parameters did not show a normal distribution, two non-parametric ranking tests were used. The Mann–Whitney U-test is never less than 86 % efficient for detecting differences in location of populations with the same shape of distribution (Elliott, 1971), while the Kolmogorov–Smirnov test is a more powerful test when distribution shapes differ.

Results

Tension reduction by FETi inhibition

Procedure, and the problem of experimental controls

To determine the effects of current inhibition of FETi, the experimental kicks have to be compared with control kicks. However, individual locust kicks come in a wide variety of motor patterns within an overall programme consistency, and so it is not possible to predict exactly what pattern an experimental kick *would* have displayed had current not been injected. We show paired experimental and control kicks in which the motor pattern of the control kicks approximately matches that of the experimental kicks before (and sometimes after) current injection. The control kicks thus show that motor programmes similar to those in the experimental kicks *can* occur, but they are *not* predictions of what the experimental kicks would have looked like had current not been injected (that clearly begs the question). By showing actual data we are able to illustrate characteristics of the motor programme which are difficult to extract numerically. However, because of the restriction in the number of records it is feasible to show, there is a danger of choosing unrepresentative samples. To try to obviate this, our second approach was to tabulate the dominant characteristics of 30 consecutive kicks from a single preparation, in which 15 were the controls and 15 were experiments with current inhibition.

Terminal inhibition

Fig. 2A shows a control kick with an initial flexion, indicated by low-frequency FlTi activity, followed by co-activation of FlTi and FETi motor neurones. The FlTi motor neurone shows a massive increase in spike frequency at the time of the first FETi spike, and continues to spike at high frequency until the trigger activity terminates its burst. Tibial extension occurs 70 ms after flexor inhibition. FETi fires an initial high-frequency burst, and there is then a slight decline in frequency, followed by an increase leading to a peak frequency of spiking (the terminal burst) just before the trigger inhibition of the flexor. This illustrates the motor programme typical of a fairly powerful kick. In Fig. 2B a current-inhibited kick is shown. There is a very similar bout of initial FlTi activity, followed by co-

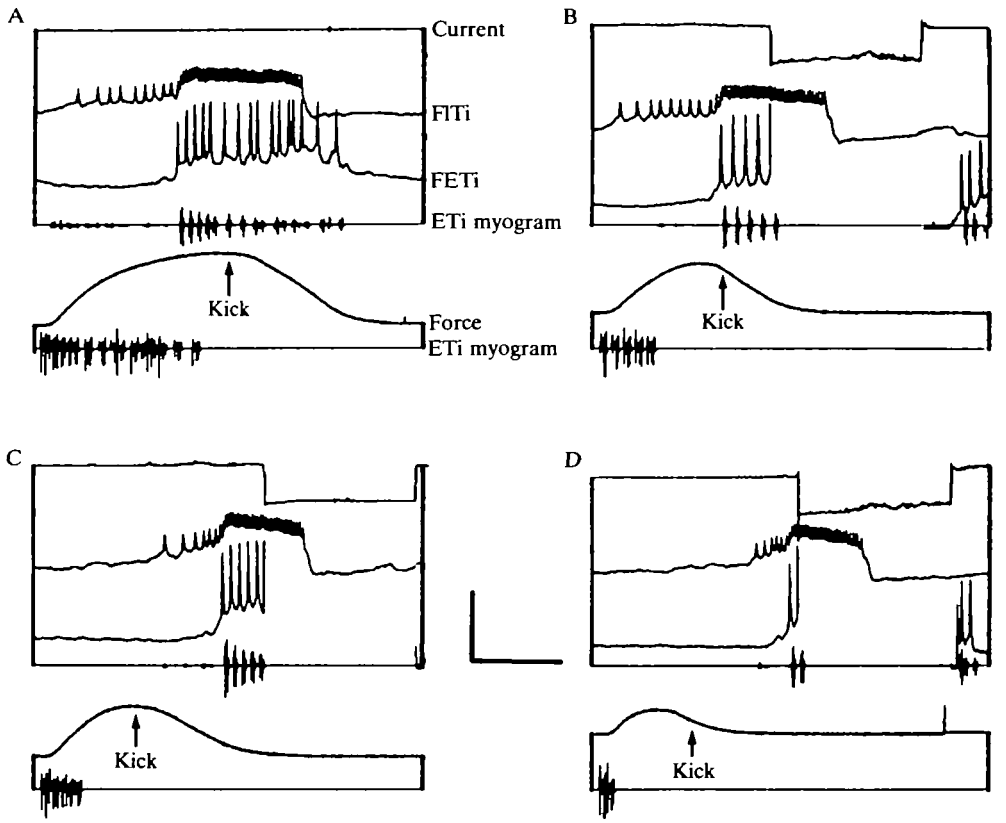

Fig. 2. (A–D) Current inhibition of FETi for the terminal part of co-contraction. (A) A normal kick with no current inhibition. (B–D) Kicks in which FETi is inhibited progressively earlier in co-contraction. The upper set of bracketed records show the current monitor (top), intracellular recording from FlTi motor neurone (second), intracellular recording from FETi (third), myogram of ETi and some FlTi units (bottom). The lower set of bracketed records show tension (top) and myogram (bottom) recordings made by stimulating the axon of FETi in another preparation with the same pattern of FETi spikes as shown in the upper records. In this and Figs 3 and 4 the arrow on the tension record shows the estimated time of tibial extension. Scale: vertical 20 mV, 200 nA, 12 g; horizontal 200 ms.

activation of FETi and FlTi, with a similar increase in FlTi activity coincident with the first FETi spike. However, FETi is current-inhibited 125 ms after it starts to spike. There are no further FETi spikes, but the FlTi motor neurone continues spiking at high frequency until it undergoes trigger inhibition. The tension records show a surprisingly long latency between the myogram spikes and tension development. Nonetheless, the tension at the time of tibial movement in the experimental kick was reduced to 70 % of that of the control kick. Despite this difference in tension, the FlTi activity is very similar in the control and experimental kicks.

Two further kicks with terminal current-inhibition are shown in Fig. 2C,D. The initial flexion is less strong (fewer FlTi spikes) than in Fig. 2A,B, but there is still a large increase in FlTi spike activity coincident with the first FETi spike. In Fig. 2C FETi fires five spikes before it is current-inhibited, while in Fig. 2D only two FETi spikes occur. There is a considerable difference in the ETi tension developed by the two kicks. Despite this, the durations of the high-frequency FlTi bursts are virtually identical, and there is obvious trigger activity even when ETi tension is minimal. There is, however, a slight decline in FlTi spike frequency and a negative shift in membrane potential towards the end of the burst which is not usually seen in the control kicks.

Initial inhibition

In some experiments current inhibition was fortuitously applied before co-activation began. Fig. 3A shows a control kick with a fairly powerful initial flexion phase. As usual there is a dramatic increase in FlTi activation coincident with the first FETi spike. Fig. 3B shows an experimental kick with an approximately similar initial flexion. Current inhibition starts during the initial flexion, and FETi is prevented from spiking until about 125 ms before the trigger activity. Despite the absence of any FETi spikes the transition from initial flexion-type FlTi activity to high-frequency co-activation-type FlTi activity is clear-cut, although the initial intensity of the FlTi burst is slightly less than in the control kick. On release from current inhibition, FETi spikes at high frequency, and this is followed by an increase in FlTi spike activity. This increase has a latency of 20–30 ms, which means that although it cannot be a response to the central FETi–FlTi connection, it might be a response to the developing ETi tension. The tension at the moment of the experimental kick is 84 % of that occurring in the control kick.

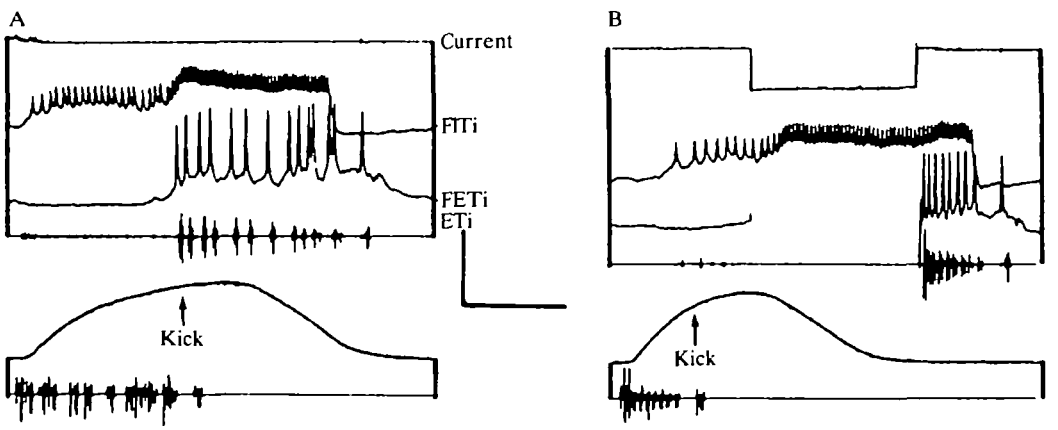

Fig. 3. (A,B) Current-inhibition of FETi for the initial part of co-contraction. (A) A normal kick with no current inhibition; (B) a kick in which FETi is current-inhibited. Traces and scale as in Fig. 2.

Total inhibition

On some occasions FETi current inhibition spanned the entire duration of a fictive kick. It is, of course, difficult to define an event in which there are no FETi spikes, no ETi tension and hence no tibial movement as a kick. However, episodes in response to stimulation in which the FlTi activity was very similar to that occurring in a real kick were recorded under these circumstances. Fig. 4A shows a weak kick, in which the co-contraction is of relatively short duration, but there is normal pre-flexion and trigger FlTi activity. It is interesting to note that, in this kick, tension has not reached a maximum at the time of tibial movement. The motor programme thus does not appear to be optimal in terms of behavioural efficacy. In Fig. 4B a similar episode of FlTi activity occurs, but FETi is current-inhibited for the entire duration of the high-frequency FlTi burst. FETi fires a few spikes on release from inhibition, but this is long after the 'trigger activity' has terminated the FlTi burst. Thus the essential features of the FlTi motor programme can occur with no FETi spikes, and thus no ETi tension at all. This particular 'kick' had a 'co-contraction' phase of rather short duration. However, this was not caused by the absence of FETi spikes, since Fig. 3B shows a kick with extended initial inhibition in which there are no FETi spikes, but normal FlTi activity occurs which is considerably longer than the total duration of Fig. 4B.

Summary statistics

Table 1 and Fig. 5 show summary data for 15 control and 15 current-inhibited kicks which occurred in a continuous sequence. In all kicks the terminal marker is the time of FlTi trigger inhibition (this was always quite unambiguous). In the control kicks, the initial marker is the first FETi spike of the co-contraction, whereas in the experimental kicks the initial marker is either the first FETi spike or, in the cases where FETi was initially inhibited, the sudden increase in FlTi

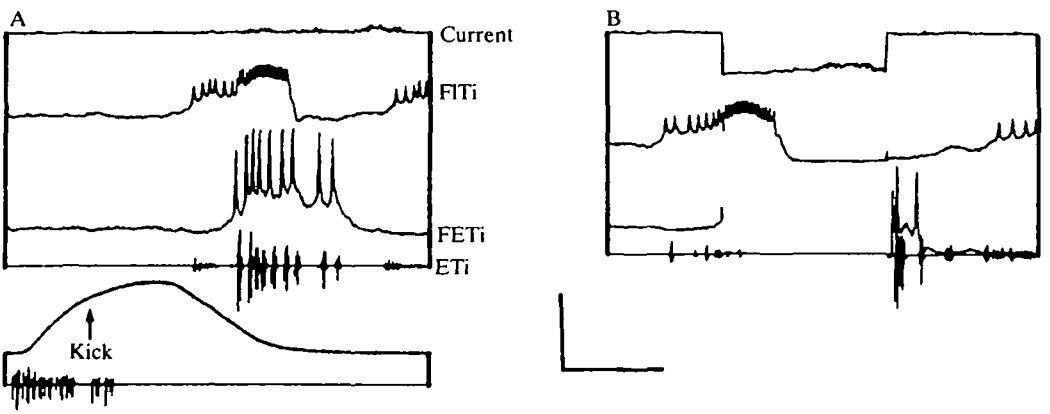

Fig. 4. (A,B) Current-inhibition of FETi for the total duration of (fictive) co-contraction. (A) A normal kick with no current inhibition; (B) a kick in which FETi is inhibited throughout the period of high-frequency FlTi spiking. Traces and scale as in Fig. 2, except no tension record is shown for B, since no tension was developed.

Table 1. *Summary statistics from a continuous sequence of 30 kicks, in 15 of which FETi was current-inhibited for varying durations (see Fig. 5)*

Measured parameter	Mean control	Mean experimental	Probability U	Probability K–S
FETi duration (ms)	246	111	<0·1	<0·1
FlTi duration (ms)	246	264	NS	NS
FETi spikes	9·27	4·40	<0·1	<0·1
FlTi spikes	32·5	32·0	NS	NS
FETi frequency (Hz)	40·5	42·5	NS	NS
FlTi frequency (Hz)	130	125	NS	NS
ETi force (g)	11·6	6·96	<0·1	<0·1
trigger (mV)	10·5	11	NS	NS

The probability column gives the probability (as a percentage) that the control and experimental data derive from the same population, measured using the Mann–Whitney U-test (U) and the Kolmogorov–Smirnov test (K–S).

Probabilities greater than 5 % are listed as not significant (NS).

frequency which usually marks the transition from initial flexion to co-contraction. FETi and FlTi spike counts are made between the markers. Any FlTi spikes occurring prior to the first FETi spike are regarded as belonging to the initial flexion phase of the behaviour. Control FETi and FlTi burst durations are the time between the markers. Experimental FETi burst duration is the interval between these markers during which FETi was not current-inhibited. Thus FETi and FlTi durations are identical for the controls, but differ in the experimental kicks. Relative force was measured in a different preparation by stimulating the FETi axon with the appropriate pattern. The data refer to force at the end of the tibia at the moment of movement, or 60 ms after the trigger activity in episodes when movement could not be detected.

Not surprisingly, there is a highly significant difference between control and experimental kicks with regard to FETi burst durations, FETi spike counts and force measurements. The data are presented in Fig. 5, visual inspection of which shows that these three parameters are highly positively correlated. None of the other measured parameters showed a significant difference between experimental kicks and controls. The lack of correlation between current inhibition and FETi frequency suggests that there was no significant post-hyperpolarizing rebound or excess excitation of FETi to 'compensate' for the inhibited period. There is no significant difference between experimental and control kicks for FlTi duration, spike count, frequency or trigger inhibition (although there is a slight trend for a decrease in the number of FlTi spikes per kick and the amplitude of the trigger activity over the sequence). Thus the changes in FETi central activity, and the consequent changes in ETi tension, have no measurable effect on the motor programme controlling the FlTi motor neurone. If FETi-inhibition does indeed slightly weaken the concurrent FlTi burst during co-contraction (an impression

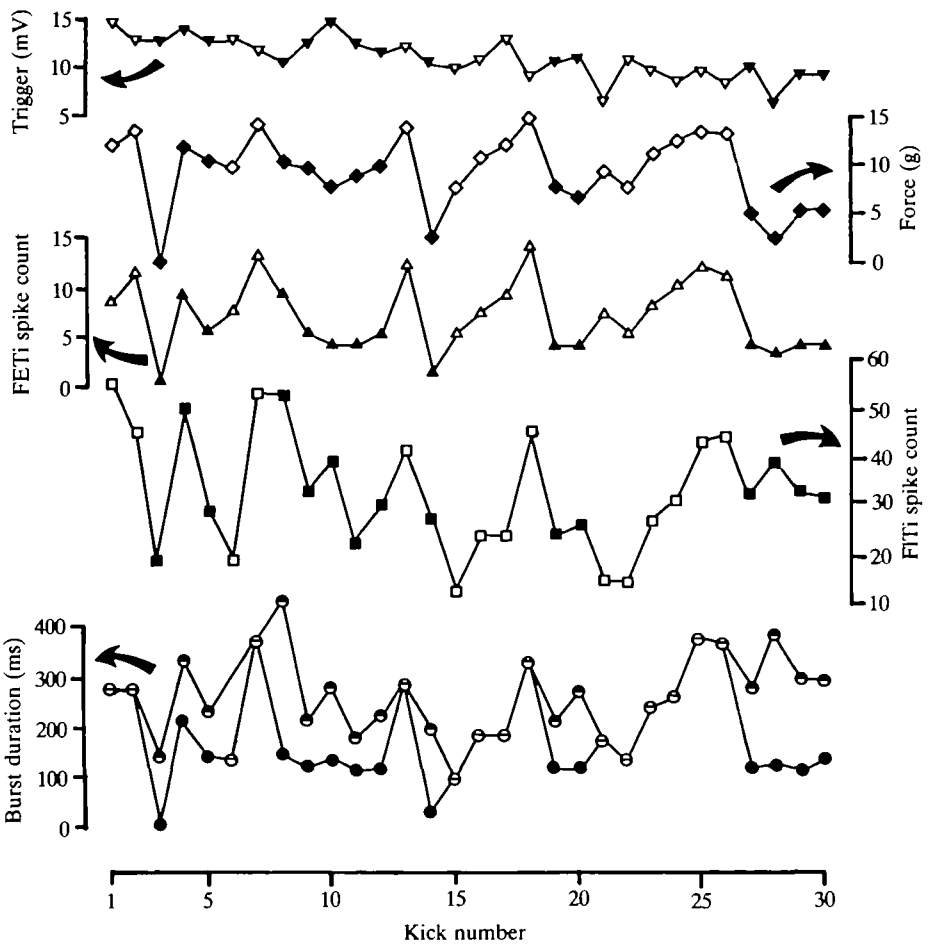

Fig. 5. Summary of data from 30 consecutive kicks, 15 experimental kicks with current inhibition of FETi (solid symbols), and 15 control kicks with no inhibition (open symbols). In control kicks the FETi and FITi durations are identical (open circles with horizontal lines). In experimental kicks FETi burst durations (solid circles) are reduced, while FITi burst durations (half-solid circles) are not significantly changed. FETi spike counts (upright triangles) and tibial extensor forces (diamonds) differ significantly between experimental and control kicks, but FITi spike counts (squares) and FITi trigger activity amplitudes (inverted triangles) do not. See Table 1 for more details.

gained by visual inspection of some of the records), the effect is too small to be detectable by these analytical techniques.

Tension reduction by nerve section

Procedure

To try to minimize problems caused by the inherent variability of the motor programme, a standard experimental regime was used. At the start of the

experiment a 'fake' nerve cut was performed. This was identical to the operation used to cut the ETi nerve (see Materials and methods), except that the nerve was not cut. This was so that any effects on the kick motor programme resulting from the operation trauma (rather than from the nerve section itself) would be discounted in the following pre-cut controls. We waited 15 min to allow recovery from any short-term disruptive effects of the operation, and then attempted to elicit about 10 control kicks (series 1). Next, ETi tension was measured in response to standard stimuli applied to the FETi axon. The ETi muscle was then partially denervated by nerve section, and an identical stimulus regime applied to determine the drop in ETi muscle tension. A further 15 min recovery period was allowed following the cut before we attempted to elicit 10 experimental kicks (series 2).

Two sorts of control experiment were carried out. In the first, no operation at all was performed on the femur, but kicks were induced with the same temporal regime. Tension was measured before, between and after the two bouts of kicks. This was to determine whether there were any trends in kick performance or muscle tension simply associated with ageing of the preparation. In the second controls, exactly the same procedure was carried out as in the experiments, except that the 'cut' made between the two bouts of kicking was again fake. This was to determine whether the small but inevitable disruption of the femur associated with a second 'fishing expedition' for the ETi nerve produced a change in ETi tension or motor programme, irrespective of the nerve section.

Tension

Nerve section produced an average drop in ETi tension of 42% ($N = 8$, S.D. = 8·7, range = 32–57) in response to stimulation at 34 Hz, and a drop of 57% ($N = 8$, S.D. = 8·8, range = 47–75) in response to single stimuli, as measured immediately before and after the cut. There was no perceptible change in tension accompanying the 'fake' nerve cuts (six experiments). There was sometimes a slight drop in tension (5–10%) between the beginning and the end of an entire experimental run (lasting 1–2 h), but on other occasions there was no perceptible change.

Analysis of kicks

Nerve-cut experiments were carried out on 12 animals, but in many cases the full procedure could not be applied because of reluctance of the animal to produce sufficient kicks. We present data from three experimental animals and three of each of the controls.

The exact structure of motor activity within co-contraction can be complex, with several sub-bursts occurring within the main FETi burst. We have not attempted to quantify these structural aspects, but visual inspection of the records shows no obvious consistent differences between kicks elicited before nerve section in which the full ETi tension was developed, and kicks elicited after section of the nerve when tension had been reduced by about one-third (Fig. 6). In particular, the

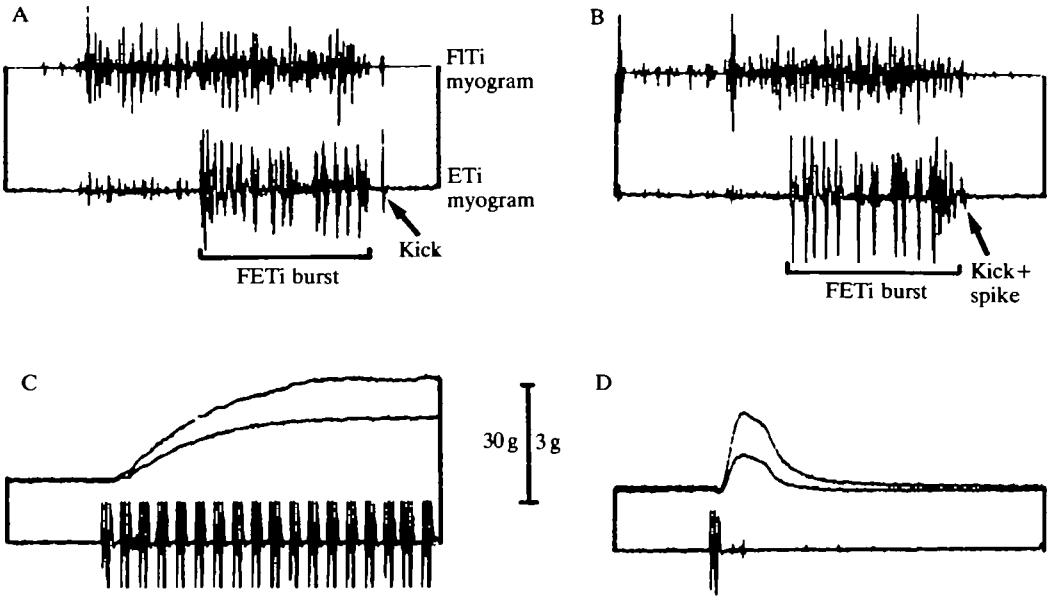

Fig. 6. (A–D) Effects of partial ETi muscle denervation on motor programme and ETi muscle tension. (A) A kick showing myograms from the FlTi muscle (upper trace) and ETi muscle (lower trace) immediately prior to nerve section. The bracket shows the duration of the FETi burst. The arrow indicates the movement artefact caused by the sudden tibial extension of the kick. (B) A kick induced immediately after the recovery period following partial denervation. The bracket again shows the duration of the FETi burst. The arrow shows the last FETi myogram spike coinciding with the movement artefact. The terminal burst is clearly evident just before the kick, and the FETi myogram spikes decline in amplitude as they increase in frequency. (C) Two sweeps superimposed showing the muscle tension (upper trace) and ETi myogram (lower trace) before and after partial denervation in response to stimulating FETi at approximately 30 Hz. (D) As C, except that a single stimulus pulse is applied. In C and D the larger tension response occurred before denervation. Each record is 700 ms in duration.

terminal burst (a rapid increase in FETi spike frequency just before the moment of the kick) was clearly visible in several examples of both control and experimental kicks.

The duration of co-contraction (time from the first FETi spike to the last before the movement) and the total number of FETi spikes were measured for each kick. From these the average frequency of FETi spikes was calculated (Table 2; Figs 7–9). The data show that there is considerable variability between successive kicks made by the same individual, and some, but not all individuals show trends in performance over the sequence of about 20 kicks. In the unoperated controls (Fig. 7) there is a slight trend for co-contraction to become shorter, and the spike total to fall during the first 3–4 kicks. The kicks then seem to stabilize, and there is no further obvious trend. In only one case is the decline between series 1 and 2 significant, but Fig. 7 suggests that this decline occurs within series 1 kicks, rather

Table 2. *Summary statistics from the nerve-cut experiments*

Unoperated control kicks

	FETi count			Duration			Frequency		
	A1	A2	A3	A1	A2	A3	A1	A2	A3
Mean 1	12·2	11·7	11·8	216	398	272	56	30	44
Mean 2	10·2	11·1	7·3	171	364	181	61	30	44
U-test	NS	NS	1–2·5	NS	NS	2·5–5	NS	NS	NS
K–S test	NS	NS	5	NS	NS	NS	NS	NS	NS

Operated control kicks

	A1	A2	A3
Tension ratio 2:1 single stimulus	1·0	1·2	1·0
multiple stimulus	1·0	1·0	1·0

	FETi count			Duration			Frequency		
	A1	A2	A3	A1	A2	A3	A1	A2	A3
Mean 1	9·1	12·6	11·9	275	390	332	35	32	37
Mean 2	8·8	14·8	11·4	248	399	260	36	37	44
U-test	NS	2·5–5	NS	NS	NS	NS	NS	2·5	5
K–S test	NS	NS	NS	NS	NS	NS	NS	2·5–5	NS

Experimental kicks

	A1	A2	A3
Tension ratio 2:1 single stimulus	0·40	0·40	0·43
multiple stimulus	0·53	0·60	0·68

	FETi count			Duration			Frequency		
	A1	A2	A3	A1	A2	A3	A1	A2	A3
Mean 1	15·5	12·2	15·1	362	280	238	43	44	65
Mean 2	14·2	8·5	12·9	365	212	209	39	41	63
U-test	NS	1	NS	NS	1	NS	NS	NS	NS
K–S test	NS	1	NS	NS	1	NS	NS	NS	NS

The mean parameters from unoperated controls, controls in which a fake cut were made, and experimental preparations for series 1 and series 2 kicks (pre- and post-cut in the experimental preparations) are shown.

Raw data, giving numbers and distribution, are shown in Figs 7–9.

Mean frequencies were calculated and tested from the raw data, and so do not exactly match those which would be derived from the mean counts and durations.

Probabilities (as percentage) that series 1 and series 2 parameters derive from the same population, measured using the Mann–Whitney U-test (U), and the Kolmogorov–Smirnov test (K–S), are given below the means.

Probabilities greater than 5 % are listed as not significant (NS).

than at the transition. FETi frequency remains approximately constant throughout. In the operated controls (Fig. 8), there is no obvious initial decrease, but kick performance declines just prior to the fake cut. Following the fake cut both duration and FETi spike count increase for a while, but then decline again. The

change at this point might be due either to recovery from fatigue or to arousal resulting from the stimulation associated with the fake cut (or both). There is no overall significant change in duration, but in one case there is a significant increase in spike frequency. In the full experiments (Fig. 9), where the real cut was

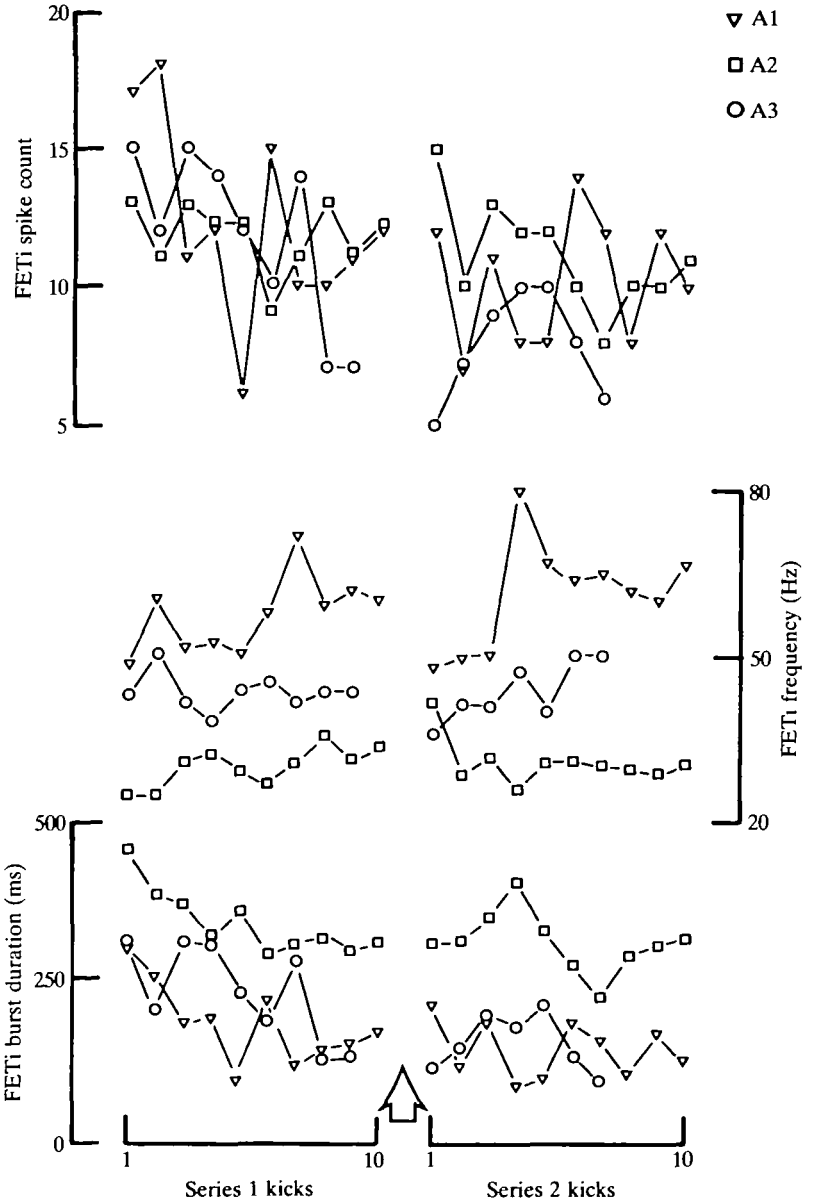

Fig. 7. Summary of data from unoperated control kicks, showing the number of FETi spikes in the co-contraction burst (upper graph), the mean FETi frequency (second graph), and the duration of co-contraction (lower graph). No operation was performed between series 1 and series 2 kicks. See Table 2 for more details.

made, there is no such pre-cut decline. Since prior to the cut the experimental and operated control preparations have had identical treatment, the decline seen in the operated controls cannot be regarded as a consistent feature. There is no change in duration or spike count in two of the experimental preparations following the real

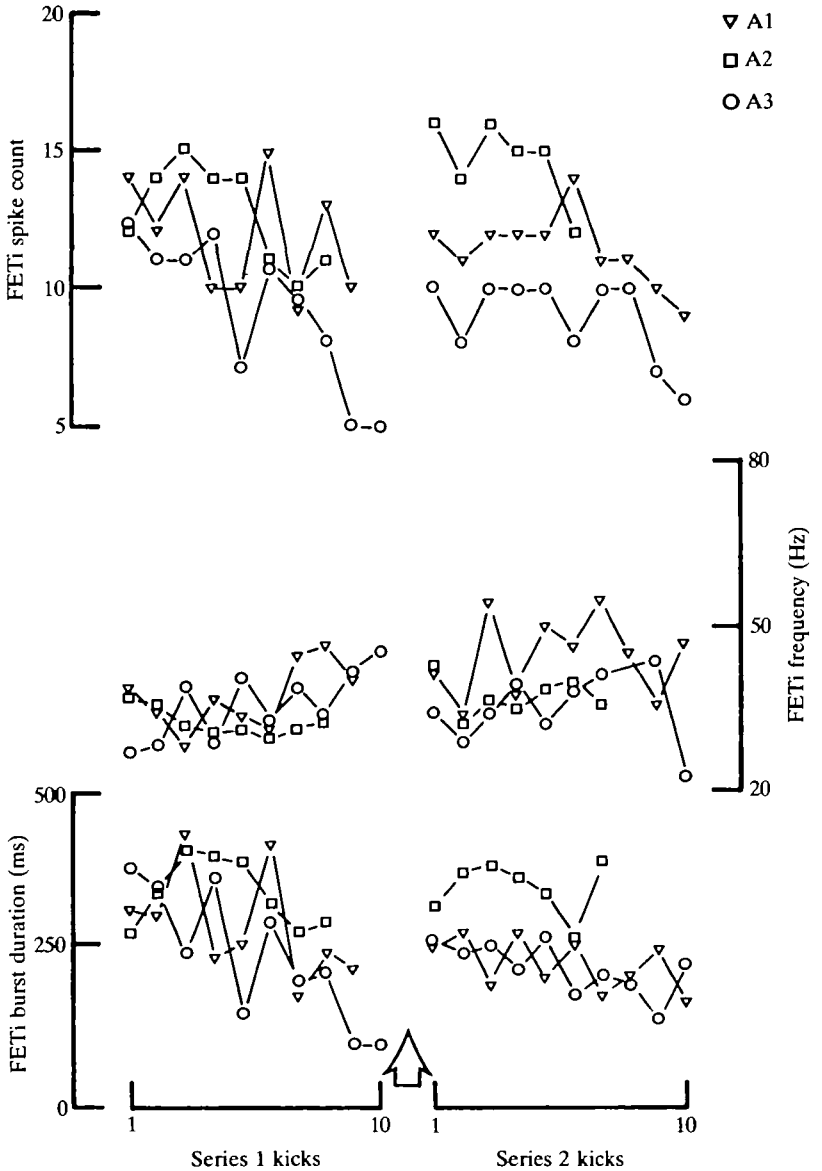

Fig. 8. Summary of data from operated control kicks, showing the number of FETi spikes in the co-contraction burst (upper graph), the mean FETi frequency (second graph), and the duration of co-contraction (lower graph). A fake nerve-cut operation was performed between series 1 and series 2 kicks. See Table 2 for more details.

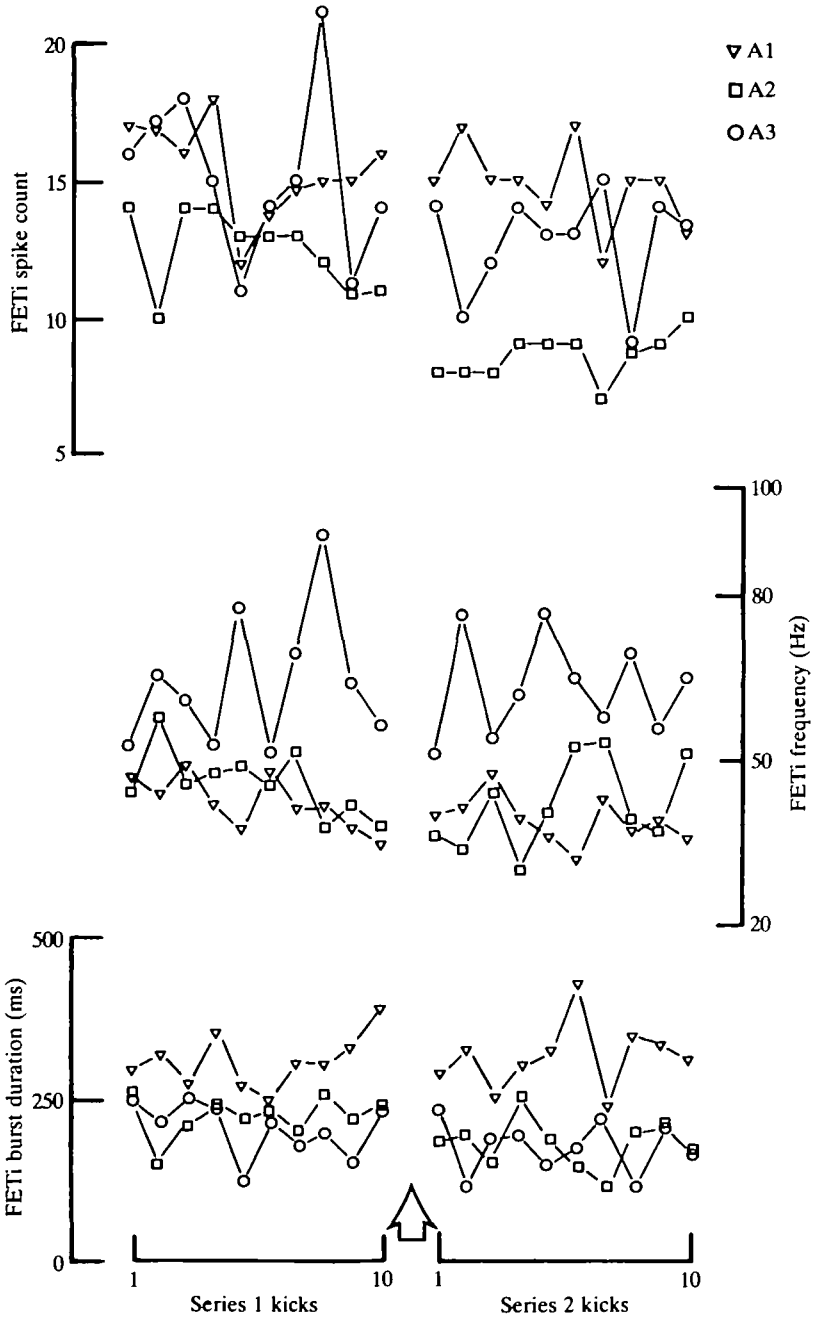

Fig. 9. Summary of data from experimental kicks, showing the number of FETi spikes in the co-contraction burst (upper graph), the mean FETi frequency (second graph), and the duration of co-contraction (lower graph). A nerve-cut operation was performed between series 1 and series 2 kicks. See Table 2 for more details.

cut, but in the third (square markers, A2) there is a significant decline in both parameters. There is no significant change in FETi frequency in any of the three experimental preparations.

Discussion

The purpose of this research was to test the hypotheses outlined in the Introduction concerning the role of FETi activity in the circuitry underlying the kick motor programme. One feature that emerges very clearly from the results is that although kicks all have the same basic motor programme, they are extremely variable in detail. This means that the effects of experimental perturbations of the programme are difficult to quantify, since it is hard to predict what parameters a particular kick *would* have had in the absence of the perturbation. We therefore chose different experimental strategies to approach the same problem.

The current inhibition experiments have the advantage that they are reversible, so that control and experimental kicks can be interspersed, thus discounting any trends in kick performance over time. Furthermore, precise measurements of motor activity, including subthreshold activity, can be made for the recorded neurones. Because FETi is inhibited centrally, an assessment of the role of its central connection to the FlTi motor neurones can be made. However, it is difficult to distinguish clearly between any effects caused by the change in FETi *central* output, and those caused by the change in peripheral feedback consequent on the change in ETi tension. Only one FlTi motor neurone was recorded in any particular experiment, but we know from previous work that all FlTi motor neurones have the same qualitative motor programme (Heitler & Burrows, 1977*a*; Gynther & Pearson, 1986).

The nerve-cut experiments have the advantage that ETi tension is affected without there being any direct effects on the central nervous system, so any changes in motor programme must result from changes in sensory feedback. Since FETi activity is not controlled directly, the central nervous system could compensate for the lost tension by increasing the drive to FETi, which should be detectable as a change in frequency. A disadvantage of this technique is that the technical difficulty of the operation precludes intracellular recordings. FETi myogram spikes can usually be reliably identified and counted, but the FlTi myogram is too complex. Thus any changes in the FlTi motor programme (other than very gross ones) are not detected with this approach. A second disadvantage is that the cut is irreversible, and so one can only compare a series of pre-cut kicks with a series of post-cut kicks. The control experiments were designed to minimize this problem by enabling us to discount any long-term trends in kick performance. We attempted a similar set of experiments in which the ETi tendon itself, rather than the ETi nerve, was cut. We hoped by this method to produce more spectacular drops in tension. However, this procedure necessitated more extensive dissection, and we were unable to obtain satisfactory control kicks.

FETi motor programme

The salient feature of the FETi motor programme during a kick is simply the occurrence of a relatively high-frequency burst of spikes with a variable duration of 150–500 ms. A positive-feedback excitatory reflex driven by ETi tension has been implicated in the maintenance of this burst. However, if FETi is prevented from spiking during the initial period of co-activation by current inhibition, it spikes at high frequency upon release, even though at that point there is no ETi tension, and hence no reflex feedback (Fig. 3B). FETi spiking occurs even when the external stimulus used to induce the motor programme terminates prior to release from inhibition, and also occurs when the kick is totally spontaneous. Current inhibition in a quiescent preparation does not lead to high-frequency post-inhibitory rebound spiking in FETi. The nerve-cut experiments, which reduced ETi tension by at least 40 %, show no reduction in FETi spike frequency such as might be expected to result from a significant loss of excitatory drive. Neither do they show an increase in frequency, such as would occur if the locust attempted to compensate for the loss of tension. In one of the three nerve-cut experiments there was a significant decrease in FETi burst duration, the reason for which is not known. It might be a genuine effect of tension reduction on the central circuitry underlying the FETi co-contraction burst, or it might simply reflect ageing of this particular preparation. In the current-inhibition experiments, where reduced-tension and normal-tension kicks were interspersed, the duration of the FlTi burst (which is normally identical to that of the FETi burst) was not changed.

FlTi motor programme

The salient features of the FlTi motor programme are the transition from the low-frequency spiking of initial flexion to the high-frequency spiking of co-contraction, the maintenance of co-contraction for a variable period from about 150–500 ms, and the sudden trigger inhibition. The current inhibition experiments clearly show that the initial-flexion to co-contraction transition is not dependent on the FETi–FlTi central connection (confirming the conclusion of Gynther & Pearson, 1986, based on timing). The intensity of FlTi spiking during co-contraction *does* appear to be slightly dependent on FETi spikes, in that visual inspection of some records reveals a reduction in FlTi spike frequency during the period of 'co-contraction' when FETi is inhibited. We suspect that this may be a genuine effect of the reduced ETi tension, but the problem of inherent variability makes it difficult to be sure. Any reduction that does occur, however, is not sufficient to be significantly reflected in a quantitative analysis of FlTi spike frequency in a larger sample of kicks. Finally, neither the timing nor the amplitude of the trigger inhibition of FlTi motor neurones is significantly affected by experimentally induced changes in FETi activity and ETi tension. Perhaps the most telling experimental result to illustrate these points is that shown in Fig. 4B, in which each of these features of the FlTi motor programme is apparent without *any* FETi spikes at all.

FETi revisited

The overall conclusion of these experiments is that the kick motor programme is generated by central mechanisms which do not rely on the effectiveness of FETi motor output (either centrally or through peripheral feedback) for their continued expression. The original hypotheses concerning the crucial importance of the various circuits involving FETi activity in the generation of the kick motor programme are thus not correct. The circuits do indeed exist, but their disruption does not lead to statistically significant changes in several quantifiable aspects of the motor programme. The circuits may augment the motor programme, but they do not control the major aspects of its expression. This is not to say that all sensory input is unimportant for the kick programme; ablation of the chordotonal organ, which monitors tibial position, normally prevents kicking (Bässler, 1968), as does physically preventing full tibial flexion (Gynther & Pearson, 1986). However, if a proprioceptive gate exists, its latch is not controlled by FETi activity or ETi tension. Since the behavioural effectiveness and presumably the evolutionary advantage of both jumping and kicking depend upon timing the release of the tibia at the point when the ETi tension is maximal, it is surprising that experimentally induced variations in tension have no effect on this timing.

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