THE INFLUENCE OF PROPRIOCEPTORS SIGNALLING TIBIAL POSITION AND MOVEMENT ON THE KICK MOTOR PROGRAMME IN THE LOCUST

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Summary

Four main proprioceptors monitor tibial position in the hindleg of the locust: the femoral chordotonal organ (FCO), the lump receptor, the suspensory ligament receptors and Brunner's organ. The influence of these proprioceptors on quantitative aspects of the kick motor programme has been investigated. The parameters measured were the duration of the initial flexion burst, the duration of co-activation of flexor and fast extensor tibiae (FETi) motoneurones, the number of FETi spikes during the co-activation, the interval between the kick and post-kick flexion, the number of FETi spikes occurring in this interval and the duration of post-kick flexion activity. The lump receptor and Brunner's organ have no detectable effect on any of these parameters. The FCO has highly significant effects on the duration of both initial flexion and post-kick flexion bursts, and on the number of FETi spikes occurring after the moment of tibial extension. The suspensory ligament receptors have significant effects upon the number of FETi spikes after the kick and the interval between the kick and the post-kick flexion. However, no proprioceptor had any influence upon the duration of co-activation or the number of FETi spikes during the co-activation. Thus, although elements of the kick motor programme preceding and following co-activation are strongly influenced by proprioceptors monitoring tibial position and movement, the co-activation stage, which is central to the effectiveness of the complete behaviour pattern, is not affected.

Key words: *Schistocerca gregaria*, grasshopper, central pattern generator, jump.

Introduction

A key to understanding the neural mechanisms producing any highly structured motor programme is to determine the relative roles of centrally programmed elements as opposed to peripheral feedback from sensory systems. To what extent is the motor programme 'hard wired' so that, once initiated, it follows a pre-determined course, and to what extent does the central nervous system monitor the progress of the programme using sensory feedback and adjust its output accordingly? This type of question has been investigated quite extensively in rhythmic systems (Forssberg *et al.* 1977; Hooper and Moulins, 1989; Lennard and Hermanson, 1985; Möhl and Nachtigall 1978; Pearson, 1972), but is relatively unexplored in episodic behaviour patterns.

The locust jump or kick is a type of episodic behaviour that can push the mechanical system of the hindlegs (muscles and skeleton) to the performance limits of biological materials. To achieve this requires a multi-stage motor programme (Heitler and Burrows, 1977*a*), in which each stage has to be carried out in the correct sequence and with the correct timing. The first stage is the *initial flexion* of the hindlegs. This stage is essential, because only in the fully flexed position does the geometry of the femoral–tibial joint allow the relatively weak flexor muscle to hold the tibia against the increasing extensor torque which develops in the second stage. The second stage is *co-activation* of the flexor and extensor muscles. The flexor muscle keeps the tibia fully flexed, while the extensor muscle slowly contracts to achieve maximum force. Energy is stored in distortion of the semi-lunar processes (Bennett-Clark, 1975). The third stage is the *trigger* inhibition of the flexor motor system, releasing the tibia from the flexed position and allowing it to extend, utilising the stored energy. There is usually a fourth stage, consisting of *re-flexion* of the tibia, preparatory to landing or producing another kick, but this stage may be absent.

The potential 'do-or-die' aspect of the defensive kick and escape jump of the locust suggests that there may have been considerable evolutionary pressure to produce an optimal synergy between the central and peripheral components of the motor programme, and so the system provides a good opportunity to explore this balance. In a previous study (Jellema *et al.* 1997), we showed that the summed input from three sensor groups that monitor tibial position has a strong influence on the probability of the switch from the first, initial flexion, stage to

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the second, co-activation, stage of the motor programme and, hence, on the probability of occurrence of the complete programme. The sensor groups are the femoral chordotonal organ (FCO; Usherwood *et al.* 1968), the lump receptor (récepteur ventro-postéro-latéral; Coillot and Boistel, 1968, 1969; Heitler and Burrows, 1977*b*) and Brunner's organ (Uvarov, 1966; Heitler and Burrows, 1977*b*). However, it was not clear from the previous study whether these proprioceptors also affect quantitative aspects of the full motor programme when it does actually occur, and this is the topic on which we currently report. One question of particular interest is whether they affect the parameters of co-activation. The FETi activity during the co-activation stage determines the force with which the tibia extends (Burrows, 1995) and, hence, the effectiveness of the behaviour pattern, and so from an engineering viewpoint it might be expected to be subject to proprioceptive monitoring and control. The underlying question is whether co-activation is purely centrally driven so that, once initiated, its characteristics do not depend on peripheral feedback or whether peripheral feedback from tibial position helps to sustain co-activation (we do not here consider proprioceptors other than those monitoring tibial position). The findings we describe strongly support the central drive hypothesis, but also show that aspects of the kick motor programme preceding and following the co-activation stage are influenced by specific proprioceptors.

Materials and methods

Adult locusts (*Schistocerca gregaria* Forskål) of either sex were taken from a crowded colony. The animals were restrained on their backs in Plasticine, with the femur of the right hindleg firmly embedded while leaving the tibia free to move. The tarsus and distal 2 mm of the tibia of this leg were removed. The left hindleg was removed. When aroused, such a restrained locust may readily perform a defensive kick. Kicking and jumping are produced by the same three-stage motor programme (Pflüger and Burrows, 1978). Flexor and extensor electromyograms (EMGs) were recorded from the femur of the hindleg with pairs of 50 µm diameter copper wires, insulated except at their tips. Tibial movements were monitored using a photoresistor. In some experiments, a flag was attached to the tibia to increase the effective occlusion of the detector.

Parameters of the motor programme

We have analysed six parameters associated with the kick motor programme (Fig. 1A).

Duration of initial flexion

The initial flexion stage is evident in the flexor myogram traces as a burst of activity preceding co-activation. In most cases, the duration of the flexion burst could be determined unambiguously, but where there was extensive ongoing flexor activity, a gap greater than 100 ms was regarded as the defining separator of the initial flexion from any preceding flexor activity (see, for example, Fig. 1A). If background activity was present in the flexor EMG recording, the flexor burst was considered to start at the point where there was an obvious increase in flexor activity. All kicks are preceded by full tibial flexion, although this may sometimes occur before the initial flexion burst as we define it for the purpose of analysis in this report.

Duration of co-activation

The co-activation period is the period during which both the flexor motoneurones and FETi are spiking, and thus it corresponds to the interval between the first spike of the FETi burst and the trigger inhibition of the flexor tibiae motoneurones. The data for these experiments derive from EMG recordings, in which it is not possible to determine the timing of the trigger activity with absolute accuracy. However, tibial extension normally follows the trigger activity with a relatively fixed latency of approximately 60 ms (Heitler and Burrows, 1977*a*; Burrows, 1995), and so we have used the time interval between the first FETi spike and the moment of tibial extension as an operational definition of the co-activation period.

Number of FETi spikes during co-activation

FETi spikes can usually be identified unambiguously because of their large amplitude in the myogram recording. Only kicks with at least three FETi spikes during the co-activation were analysed.

Number of FETi spikes after the kick

Normally, FETi does not produce many spikes after the tibia extends in a kick (extension itself only takes approximately 20 ms). However, occasionally it does spike at this time, and these spikes were counted.

Interval between kick and post-kick flexion

In most cases, the kick was followed, after a delay, by a flexor burst which brought the tibia back into a flexed position (although not necessarily the fully flexed position). The interval between tibial extension and the start of this flexor burst was measured. Only kicks in which flexion occurred within 3 s of tibial extension were analysed.

Duration of post-kick flexor burst

The duration of the re-flexion burst was measured using the same criteria as for the initial flexion burst.

Assessing the contributions of the different proprioceptors

To assess the influence of each of the proprioceptors on these parameters, up to 15 kicks per animal were evoked using a standardised tactile stimulation regime, with the proprioceptors exposed but intact, to provide baseline levels for each of the parameters. After perturbation of one or a combination of the proprioceptors, the stimulation regime was repeated. The before- and after-perturbation parameter values were then compared. The perturbation and location of the proprioceptors and the stimulation regime have been described previously (Jellema *et al.* 1997) and are outlined below.

The femoral chordotonal organ (FCO)

The FCO signals movement and position of the tibia

(Usherwood *et al.* 1968). The experimental perturbation (treatment T) consisted of cutting the tendon linking the FCO to the tibia, causing the tendon to move into the position corresponding to partial tibial extension. After being cut, the tendon no longer responded to movement of the tibia, and thus the main mechanical stimulus to the FCO was removed. The small ventral ligament that connects the FCO to the apodeme of the flexor tibiae muscle, and the flexor strand of the FCO, were still intact, and these would cause some mechanical

perturbation of the FCO when the tibia moved. However, this would be minor in comparison with that mediated by the tendon, and thus the resulting FCO activity, if present, would be very different from the activity evoked in the fully intact preparation.

Receptors of the lateral nerve

Two groups of proprioceptors have axons in the lateral nerve. The lump receptor is an internal femoral receptor that

Fig. 1. The effect of hindleg proprioceptors on parameters of the kick motor programme. (A) A typical example of the kick motor programme as revealed by recordings from the extensor (Ext.) and flexor (Flex.) tibiae muscles. The programme can be divided into consecutive phases (indicated at the top). Quantitative descriptions of these phases were used in the analysis. The moment of tibial extension in a kick is indicated by an open arrow. The lower trace shows the tibial position as indicated by a photoresistor. (B) Illustration of the significant effects produced by treatments T and L (see text for details of treatments). The three panels on the left-hand side belong to treatment T, those on the right-hand side to treatment L. A kick recorded in the control situation (pre-treatment) and two kicks recorded after the treatment was applied (post-treatment 1 and 2) are shown for each condition. For treatment T, the increased durations of initial flexion and post-kick flexion are obvious. For treatment L, the increase in the interval between tibial extension and reflexion is also clear. Two examples (asterisks) of the occurrence of fast extensor tibiae (FETi) spikes after the kick are visible for treatments T and L. The horizontal bars indicate the duration of the co-activation. The kicks are aligned in time (indicated by dotted line) to ease comparison. The lower trace shows the tibial position (not available for the post-treatment 2 kick in treatment T); a flag was not attached to the tibia so that the fast tibial extension in a kick is now indicated by a fast vertical inflection and the subsequent slow post-kick tibial flexion by a slow inflection. In the pre-treatment panel of treatment T, the inflection indicating the kick is missing, but the corresponding artefact in the EMG recording is clear.
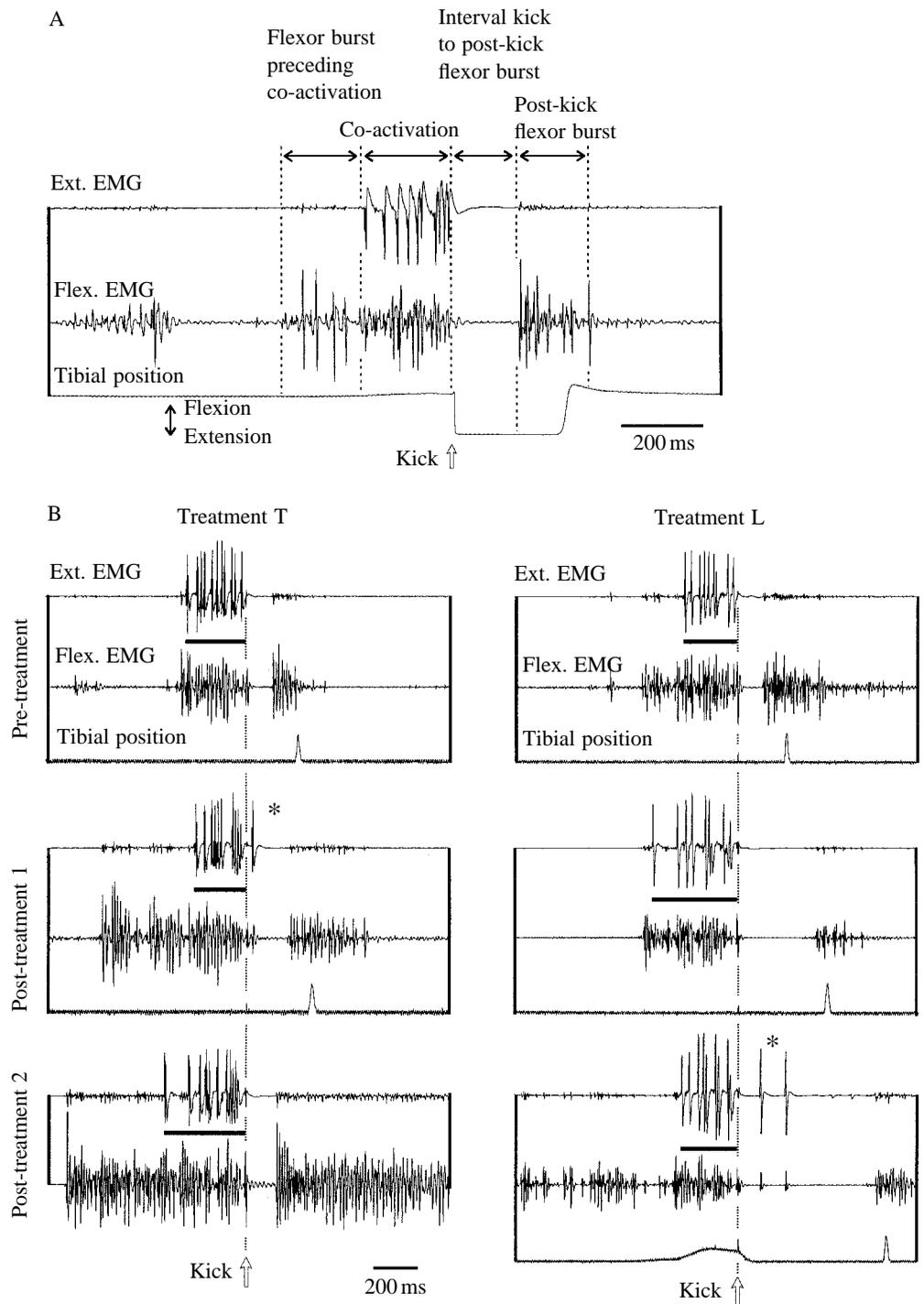
is activated when the tibia is fully flexed and there is tension in the flexor tibia tendon (Heitler, 1974; Heitler and Burrows, 1977*b*). The suspensory ligament receptors signal tibial positions from approximately 60° relative to the femur to full extension and are silent at angles smaller than 60° (Coillot and Boistel, 1969). The input from both the lump receptor and suspensory ligament receptors was eliminated by cutting the exposed lateral nerve (treatment L).

Brunner's organ

Brunner's organ consists of a small external tubercle on the ventral surface of the femur. When the tibia is fully flexed, it presses upon the tubercle, thereby activating sensory cells located just proximally (Uvarov, 1966; Heitler and Burrows, 1977*b*). Perturbation of Brunner's organ consisted of cutting a small piece from the tibia so that the fully flexed tibia was just too short to press on the tubercle (treatment B).

The experimental design

The three sensory perturbations were applied in all possible combinations, resulting in seven experimental treatments: T, L, B, T+L, T+B, L+B, T+L+B. Each animal received only one treatment. The control condition consisted of the 'fake' versions for treatment T+L+B (for details, see Jellema *et al.* 1997). Tactile arousal stimuli were applied by stroking various body parts with a fine brush. Animals that produced fewer than three kicks after the perturbation were not analysed. The effects of the perturbations on the parameters were expressed as the fractional change of the parameter value (before-treatment value divided by after-treatment value). When considering the number of FETi spikes after the kick, 0.1 was added to both before-treatment and after-treatment values prior to the division to avoid the possibility of dividing by zero in cases where no FETi spikes occurred after the kick.

Data analysis

The GLIM computer programme was used to fit a generalised linear model (Poisson error distribution, logarithmic link function and estimated scale parameter) for the before- to after-treatment ratios, in terms of the three types of applied treatment (T, L and B). A detailed description of the technical basis and procedures of this analysis has been given previously (Jellema *et al.* 1997), but a *résumé* of the underlying concept is given here. The GLIM programme was used to derive a series of 'treatment effect factors' that provide the best fit to the following equation:

$$\text{ratio} = e^{E_t T + E_l L + E_b B + E_c},$$

where the ratio is the before- to after-treatment ratio of a particular parameter of the motor programme (i.e. the experimental result), E_t, E_l and E_b are the effect factors of the treatments T, L and B respectively, *T*, *L* and *B* are either 1 or 0, depending on whether the treatment was applied (1) or not applied (0), and E_c is the effect factor of the control experiments for that parameter. The input to the GLIM programme consisted of the before- to after-treatment ratios for each of the 39 experimental animals, each paired with a code

value which indicated the particular type of treatment (T, T+L, etc.) applied to that animal. The output of the GLIM programme consisted of the values of E_c, E_t, E_l and E_b, and the standard error associated with each of these values. This was repeated separately for each of the six motor programme parameters measured.

The point of this analysis is not to derive an equation to predict the result of a particular combination of treatments, but rather to determine which of the treatment effect factors contribute significantly to determining that result. It is clear from the equation that, if all the effect factors were 0, then the ratio itself would be 1 ($e^0=1$), i.e. there would be no difference between the before-treatment and after-treatment measurements. The extent to which an effect factor deviates from zero gives a measure of the contribution of that particular treatment in producing a change in that particular parameter of the motor programme. Whether an effect factor differs significantly from zero is determined by comparing the value of the factor with the standard error of the factor; if the value of the factor is more than twice its standard error, then it differs significantly from zero. A positive treatment effect factor means that the measured parameter decreased after the treatment (the before-to-after ratio is greater than one), while a negative factor means that the parameter increased after the treatment.

There are several advantages to this method of analysis over more 'standard' procedures such as analysis of variance. It is quite insensitive to the form of the data, which do not have to be normally distributed. It takes into account all the data, so that data from combined treatments (T+L, T+B, L+B) are included in the analysis, and the effects are apportioned between the individual T, L and B treatments so as to produce the best overall fit. (We also looked for non-additive effects of combined treatments, i.e. effects over-and-above those produced by simple linear combination of the individual treatments. This was achieved by including additional effect factors for the combined treatments, E_{tl}, E_{tb} and E_{lb}, in the equation. However, the data were not adequate to support analysis of so many parameters, and so this approach was not pursued.) Finally, the method takes into account any time-dependent changes in the measured parameter, which are apportioned into the control effect factor, so that these are discounted from the treatment effects themselves.

Results

Tactile arousal stimuli applied to the restrained locust may activate the kick motor programme, resulting in the rapid and powerful extension of the tibia in a kick. Fig. 1A shows EMG recordings from the two main muscles involved in producing a kick, i.e. the extensor and flexor tibiae muscles, to illustrate the stages of the kick motor programme. Some of the parameters of the motor programme in which we were interested are indicated.

All of the 76 animals initially used produced kicks in response to tactile arousal stimuli prior to subjecting them to

an experimental or control procedure. However, some of the sensory perturbations seriously reduced the probability of kicking (Jellema *et al.* 1997). For instance, after treatment T+L+B, no kicks could be evoked, which eliminated this condition from the analysis. After other treatments, some individuals kicked, whereas others failed to do so. We analysed seven animals in each of the single perturbation conditions (T, L and B) and in the control condition, and these animals all gave at least three kicks after treatment. In the combined perturbation conditions, kicking was more seriously hampered: only four animals kicked in the T+L condition, six in the L+B condition and just one in the T+B condition (two animals in the latter condition were discarded because they kicked fewer than three times after treatment). This brought the total number of animals analysed to 39 and the total number of kicks to 498.

Table 1 shows the results of the GLIM analysis and indicates which sensory perturbations produced significant effects on the various motor programme parameters. Treatment B had no significant effect on any parameter. Treatment T had significant effects on parameters before and after co-activation whereas treatment L had significant effects only after co-activation, but neither had a significant effect on co-activation itself (Fig. 1B). These results are described in more detail in the following sections. Table 2 gives a full listing of the mean values for each parameter before and after the treatment.

Effect of sensory perturbation on kick motor programme parameters

Duration of initial flexion

Cutting the FCO tendon (treatment T) produced a highly significant increase (*P*<0.01) in the duration of the flexor burst

preceding the co-activation. No other treatment had a significant effect on this parameter. Cutting the FCO tendon causes it to move into the position appropriate to partial tibial extension, and thus presumably the increased duration of the initial flexor burst results from the animal attempting to compensate for an (erroneously) perceived failure to achieve full tibial flexion. Both Brunner's organ and the lump receptor are also capable of signalling full tibial flexion, but their signal was evidently insufficient to override the incorrect FCO signal. The loss of either or both of their respective signals with the FCO intact did not result in a detectable change in the initial flexion duration. There was no change in this parameter in control animals.

Duration of co-activation

None of the sensory perturbations had a significant effect on the duration of co-activation. This does not mean that co-activation duration was unchanged between the before-treatment and after-treatment conditions; in fact, there was a reduction in co-activation duration in nearly all animals. However, this reduction was not significantly greater in experimental animals than it was in control animals in which no effective sensory perturbation was applied. We interpret this as simply meaning that the co-activation stage of the motor programme becomes shorter over time, which fits with the subjective observation that kicks become weaker as the preparation ages. The decrease in co-activation duration was not exacerbated by the applied sensory perturbations and was thus independent of them.

Number of FETi spikes during co-activation

None of the sensory perturbations had a significant effect on

Table 1. *The results from the analysis of deviance performed by the GLIM programme, which fitted a generalised linear model to our data*

	Duration of flexor burst preceding co-activation			Duration of co-activation			Number of FETi spikes during co-activation		
	Estimate	S.E.M.	P	Estimate	S.E.M.	P	Estimate	S.E.M.	P
Control	0.363	0.217	0.103	0.243	0.062	0.0004**	0.269	0.075	0.0009**
T	−1.132	0.392	0.0066**	−0.095	0.078	0.230	−0.172	0.095	0.079
L	−0.413	0.285	0.156	0.038	0.067	0.570	0.049	0.080	0.544
B	−0.509	0.303	0.807	−0.126	0.074	0.100	−0.046	0.088	0.807

	Number of FETi spikes after kick			Interval from kick to post-kick flexor burst			Duration of post-kick flexor burst		
	Estimate	S.E.M.	P	Estimate	S.E.M.	P	Estimate	S.E.M.	P
Control	0.285	0.185	0.132	−0.090	0.1147	0.436	−0.017	0.086	0.842
T	−1.029	0.329	0.0035**	0.115	0.14	0.417	−0.575	0.125	0.0000545**
L	−0.899	0.265	0.0017**	−0.312	0.1289	0.0209*	−0.076	0.096	0.431
B	−0.126	−0.180	0.807	−0.102	0.1412	0.807	0.002	0.102	0.807

Each parameter of the kick motor programme investigated is shown separately.

The estimated effect factor (estimate), derived by GLIM, is shown for each of the treatments (T, L and B) and the control. Also shown is the standard error (S.E.M.) associated with the estimation of the effect factor, and the associated probability (*P*). The latter was obtained using the Minitab statistical program. The significant probabilities are indicated with asterisks: *P*<0.05; **P*<0.01.

Table 2. *The effects of each of the six treatments and the control on the parameters of the kick motor programme*

		Number of kicks	Duration of flexor burst preceding co-activation (ms)		Duration of co-activation (ms)		Number of FETi spikes during co-activation		Number of FETi spikes after kick		Interval from kick to post-kick flexor burst (ms)		Duration of post-kick flexor burst (ms)	
			Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Control	Pre	45	142.59	43.68	298.77	31.53	11.73	1.65	0.54	0.26	230.41	27.31	225.65	14.68
(N=7)	Post	47	145.36	39.76	242.47	30.63	9.21	1.47	0.35	0.12	234.64	19.09	243.12	16.25
T	Pre	42	120.75	17.85	236.66	19.00	9.52	1.02	0.15	0.08	255.57	54.97	247.09	31.72
(N=7)	Post	32	332.11	49.76	201.36	18.63	8.62	0.97	0.87	0.23	312.12	54.84	484.54	66.86
L	Pre	50	143.32	29.37	216.04	27.80	10.50	1.17	0.30	0.15	163.42	14.15	238.42	14.25
(N=7)	Post	49	217.35	34.45	162.37	14.51	7.91	0.66	0.94	0.21	304.13	41.49	274.12	21.61
B	Pre	50	175.52	56.72	217.36	20.47	10.88	1.88	0.56	0.10	205.92	20.74	273.11	40.53
(N=7)	Post	51	245.86	38.10	198.09	21.41	8.89	1.40	0.65	0.11	248.16	38.45	283.16	47.87
T+L	Pre	28	216.05	49.56	252.91	33.79	10.83	1.26	0.40	0.19	235.85	34.98	323.13	72.69
(N=4)	Post	23	758.23	290.21	231.70	28.69	10.62	1.86	1.30	0.30	253.26	21.52	540.59	72.40
T+B	Pre	8	199.35	–	198.04	–	10.60	–	0.30	–	226.86	–	255.21	–
(N=1)	Post	5	1045.2	–	237.00	–	12.87	–	0.87	–	359.33	–	526.00	–
L+B	Pre	38	183.23	59.99	253.57	34.17	10.98	0.98	0.33	0.18	184.12	12.43	286.50	28.31
(N=6)	Post	29	425.57	105.50	209.50	19.34	8.17	0.56	0.95	0.21	329.22	36.81	346.06	52.57

The entries are the averages of the means derived from individual animals.

S.E.M. is the standard error of the average of the individual means; *N*, number of animals in each treatment.

The numbers of kicks before (Pre) and after (Post) treatment are indicated.

the number of FETi spikes during the co-activation stage. Again, there was an overall decrease in the number of FETi spikes as the preparation aged, but this was not affected by the applied sensory perturbations. Since there was no significant change in co-activation duration, and no significant change in the number of FETi spikes, it is not surprising that there was no significant change in FETi spike frequency during co-activation (data compared by *t*-test, but not shown).

Number of FETi spikes after the kick

Cutting the FCO tendon (T) and the lateral nerve (L) both produced a highly significant ($P<0.005$) increase in the number of FETi spikes occurring after the moment of tibial extension and before tibial re-flexion. Taking all 320 kicks performed by unoperated animals (i.e. including pre-treatment data from experimental animals as well as the controls), the mean FETi spike count after the kick was 0.41. Taking the 121 kicks performed by animals that had received the T, L and T+L treatments, the mean FETi spike count after the kick was 1.03. These treatments also increased the actual number of kicks in which FETi spiked after tibial extension. In unoperated animals, 224 kicks (70 %) had no FETi spikes after tibial extension, while in treated animals 47 kicks (39 %) had no FETi spikes after tibial extension. Both the FCO and the

suspensory ligament receptors (which have axons in the lateral nerve) are capable of signalling tibial extension and are known to inhibit FETi (Field and Burrows, 1982; Heitler and Burrows, 1977b), so presumably it is the absence of the appropriate signal from these receptors that enables FETi to continue spiking after tibial extension. There was no change in this parameter in control animals.

Interval from kick to post-kick flexor burst

Cutting the lateral nerve (L) caused a significant increase in the duration of the interval between the kick and the start of the post-kick flexor burst ($P<0.05$). This effect must be ascribed to the suspensory ligament receptors which signal femoral–tibial angles greater than 60 °, since the lump receptor, which also has its axons in the lateral nerve, cannot be activated at extended tibial positions. It would appear that the animal is, so to speak, less convinced that the tibia has indeed extended in the absence of input from the suspensory ligament receptors. Conversely, in the absence of FCO input but in the presence of input from the suspensory ligament receptors, the interval is not affected, indicating that in this situation the latter is the most effective of the two in signalling that the leg has kicked. There was no change in this parameter in control animals.

Duration of post-kick flexor burst

Cutting the FCO tendon (T) produced a highly significant increase in the duration of the post-kick flexor burst ($P<0.001$). No other treatment affected this parameter, and there was no change in this parameter in control animals. Again, this effect presumably results from the animal failing to detect that its tibia has in fact flexed and, therefore, continuing the flexor motor drive beyond the normal duration.

Post-kick FETi spikes and the duration of co-activation

In the intact preparation, the FETi spike burst which characterises co-activation normally terminates at or close to the moment of tibial extension. Treatments T and L both produced a significant increase in the number of FETi spikes that occur after the kick. However, this increase was not uniform, but rather showed an inverse relationship with the duration of the co-activation (Fig. 2). Thus, given that treatment T or L had been applied, there tended to be more FETi spikes after the kick when the co-activation was of short duration than when it was of long duration. After applying treatment T, 18 of the 32 kicks showed 1–5 FETi spikes following the tibial extension, and the mean duration of co-activation in these kicks was 164 ms. In the remaining 14 kicks, no FETi spikes occurred after the kick, and the mean duration of co-activation was 237 ms. Testing these two groups revealed a highly significant ($P<0.001$, *t*-test) reduction in the duration of the co-activation for kicks with one or more FETi spikes after the movement. After applying treatment L, 29 of the 49 kicks showed one or more FETi spikes following tibial extension, with a mean co-activation duration of 151 ms, while in the other 20 kicks the spikes were absent and the mean co-activation duration was 177 ms. Testing these two groups again revealed a significant difference in the duration of the co-activation ($P<0.05$, *t*-test). In control animals, 13 out of 47 animals had one or more FETi spikes after the kick, and the mean duration of co-activation in these kicks was 228 ms. In the remaining 34 kicks, the mean co-activation duration was 235 ms. This difference was not significant ($P>0.4$, *t*-test). When a similar analysis was carried out on all the kicks performed by unoperated animals (including the pre-treatment data from the animals subjected to treatments), the difference in co-activation duration disappeared almost completely. The mean co-activation duration of kicks which had one or more FETi spikes after the kick was 236 ms, while the mean co-activation duration of kicks which had no FETi spikes after the kick was 238 ms ($P>0.4$, *t*-test).

The Spearman rank correlation coefficient between the number of FETi spikes occurring after the kick and the duration of the co-activation for the combined post-treatment data from treatments T, L and T+L was −0.223, which differs significantly from 0 ($P=0.014$). The rank correlation coefficient for unoperated animals was −0.076, which does not differ significantly from 0 ($P=0.41$). The data for kicks from unoperated animals and animals receiving treatments T, L and T+L are plotted in Fig. 3. The reason that the mean co-activation durations of kicks from unoperated animals are

Fig. 2. An inverse relationship exists between the number of fast extensor tibiae (FETi) spikes following tibial extension in a kick and the co-activation duration. Four kicks are shown. In the pre-treatment examples, the FETi spike burst terminates at or close to the moment of tibial extension. This is true for kicks with both relatively long- (Ai) and short-duration (Aii) co-activation. In the post-treatment examples, the FETi spike burst terminates close to the moment of tibial extension when the co-activation duration is relatively long (Bi), but FETi spikes continue after the moment of extension when the co-activation duration is short (Bii). In this case, five FETi spikes occur after the kick. Note also the prolonged duration of the initial flexor bursts in post-treatment animals. Markings are as in Fig. 1.
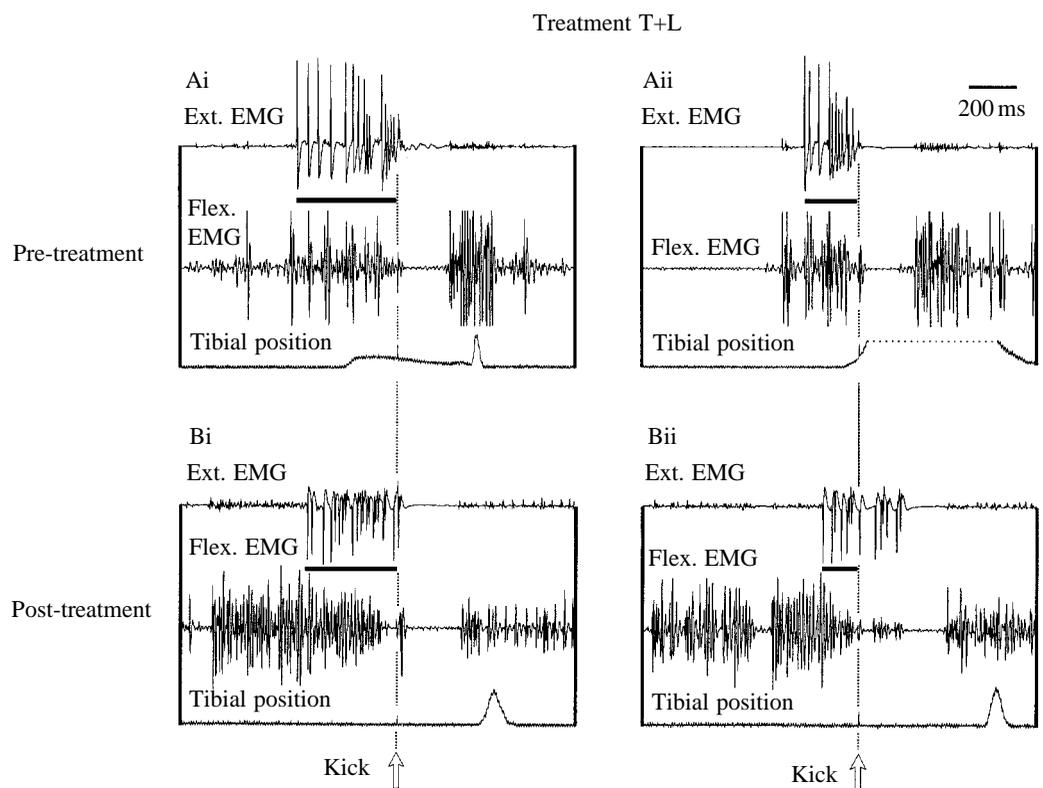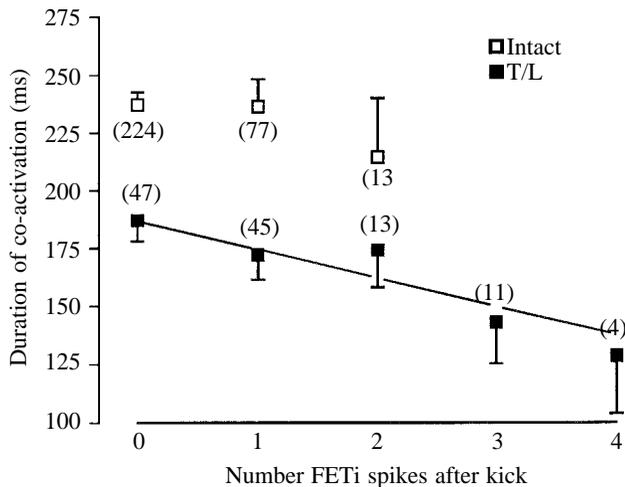
Fig. 3. The mean durations of the co-activation stage of kicks plotted against the number of FETi spikes occurring after tibial extension. Data are taken from the combined results of post-operation treatments T, L and L+T (filled symbols; see text for details of treatments) and unoperated animals (open symbols). The numbers between parentheses indicate the number of kicks from which the means were calculated. One standard error is indicated (in one direction only). A linear regression line is shown for the treatment data ($y=186-12.2x$, $r=-0.223$, $P=0.014$), but no regression line is shown for the data from unoperated animals since these showed no significant correlation. Only data points with a sample size greater than 2 are shown on the graph, but note that all results were included in the correlation and regression analysis (see text).

larger than those from the treated animals is that much of the former data derives from the pre-treatment phase of the experiment, and was thus collected earlier in the experiment, than the data from the treated animals. The data from control experiments (Table 1) show that there is a highly significant reduction in co-activation duration in the later stages of control experiments, as the preparation ages.

Discussion

This report shows that proprioceptors monitoring tibial position and movement in the hindleg of the locust play an active and specific role in various aspects of the kick motor programme. However, they do not have any detectable effect on the quantitative expression of one of the most prominent parts of the programme, the FETi output during the co-activation stage. We cannot rule out some subtle effect on, for instance, the precise pattern of flexor motor activity during co-activation, but the main feature which determines the effectiveness of the behaviour (as indicated by the force of tibial extension) is the FETi spike output (Burrows, 1995), and this was not altered by the proprioceptor perturbations we applied. We thus conclude that, while the proprioceptors monitoring tibial position play an important role in the stages before and after co-activation and are very important for the initiation of co-activation, they are not required for its

continued normal expression once it has started. We have not investigated possible proprioceptive influences from other parts of the body of the locust.

Significant effects on parameters of the kick motor programme could be detected for two of the four proprioceptors investigated: the FCO and the suspensory ligament receptors. The other two, i.e. Brunner's organ and the lump receptor, both of which were earlier shown to influence the probability of the transition from initial flexion to co-activation (Jellema et al. 1997), had no detectable effect on the motor programme parameters. The effects we detected were all consistent with the known properties of the proprioceptors involved. The extended durations of the initial flexion and of the post-kick flexion presumably result from failure of the central nervous system to receive adequate information indicating that flexion had actually occurred. The increase in the number of FETi spikes after tibial extension and the increased duration of the gap between extension and re-flexion presumably reflect a failure to detect tibial extension adequately. It is not clear why there is this particular 'division of labour' between the various proprioceptors; why, for instance, the suspensory ligament receptors but not the FCO affect the interval between kick and re-flexion, while the FCO but not the lump receptor affects the duration of initial flexion. The explanation for these differences presumably resides in the central connections made by these proprioceptors, and this awaits further investigation.

The inverse relationship that we observed between the duration of co-activation and the number of FETi spikes following tibial extension in preparations subjected to proprioceptor perturbations can be explained as follows. Previous experiments show that there exists some variation in the timing of the trigger inhibition of flexors with respect to the termination of the central drive producing the FETi burst (Heitler, 1995, Figs 4B, 5i). Furthermore, the timing of the trigger activity with respect to the start of the FETi burst (i.e. the duration of co-activation) can vary quite widely (Heitler, 1995; Burrows, 1995). Given this situation, three possibilities are evident in our experimental situation. First, if the co-activation is of long duration, then it is unlikely that the central drive to FETi will last much beyond the flexor trigger activity that releases the tibia. In this case, there will obviously be few if any FETi spikes following the kick, whatever the state of the proprioceptors. Second, if the co-activation is of short duration and the proprioceptors are intact, it is likely that the central drive to FETi will outlast the co-activation but that negative feedback from the proprioceptors will truncate the FETi output. Here again, there will be few if any FETi spikes following tibial extension (Heitler and Burrows, 1977b; Field and Burrows, 1982; Heitler, 1995). Third, if the co-activation is of short duration but the proprioceptors monitoring tibial position are ablated, then it is likely that the central drive to FETi will considerably outlast the co-activation. In this case, there will be no peripheral feedback to terminate the FETi activity and the FETi burst will continue relatively undisturbed after the tibial extension, until its drive ceases. This would account for the inverse relationship between the duration of co-

activation and the occurrence of post-kick FETi spikes following proprioceptor perturbation.

The locust kick is driven by an episodic motor programme, but it differs from other well-studied types of episodic behaviour, such as the escape tail-flip in crayfish, in that it seems to involve a much more complex level of control. In the crayfish, a brief arousal stimulus of sufficient intensity initiates a 'chain-reaction', which leads to the motor output through a relatively small number of well-defined synaptic interactions (Wine, 1984). The output follows the initiating input at very short latency (approximately 10 ms), and it is unlikely that sensory input plays any significant role in controlling the motor programme once it has been initiated. In the locust, in contrast, the latency between a brief arousal stimulus and the subsequent kick can be quite long (500–700 ms), and during this period the motor programme goes through a series of stages, some parts of which are influenced by proprioceptors, while others are not. In so far as it is a multi-stage event, the locust kick resembles a single cycle of a rhythmic behaviour pattern, and so comparison with the known effects of sensory input in fully rhythmic systems may be instructive.

At least three distinct roles have been suggested for sensory feedback in rhythmic systems (Lennard and Hermanson, 1985; Hooper and Moulins, 1989). First, in virtually all systems studied, feedback can be used to adjust the programme on a cycle-by-cycle basis to accommodate unexpected environmental perturbations. This has been demonstrated in systems ranging from flight in locusts (e.g. Möhl and Nachtigall, 1978), through swimming in tadpoles (Sillar and Roberts, 1988) to walking in the cat and cockroach (Forssberg *et al.* 1977; Pearson, 1972). In the locust kick motor programme, both the initial flexion and the post-kick flexion stages are subject to strong proprioceptive control. In the reduction or absence of sensory information indicating the completion of these stages, the motor programme either prolongs the stage in an attempt to complete it or it may revert to struggling behaviour. The proprioceptive control of these stages makes sense, because they are a part of the insect's behaviour that could easily be subject to environmental perturbation by, for example, a twig falling between the femur and tibia. In contrast, the second, co-activation, stage of the kick motor programme appears to be much less dependent on proprioceptive control, either from extensor muscle tension (Heitler, 1995) or from tibial position (this study). Even though the co-activation stage would probably not normally be subject to environmental perturbation, there is always the possibility of an internally generated error in the motor output. Since it is the co-activation stage that determines the speed and force of tibial extension, both of which are crucial for the success of the behaviour, it is perhaps surprising that there is no evidence for feedback control of this stage. However, all our proprioceptive manipulations have been static, in the sense that they exist before the start of co-activation. We have not attempted to impose a dynamic sensory perturbation during co-activation itself, and so cannot discount a possible effect in those circumstances.

The second major function for sensory input in rhythmic systems is simply to maintain and stabilise the overall level of excitability in the system. In the classic case of locust flight (historically the first demonstration of central pattern generation), the rhythm generated by the purely central mechanisms has a weaker amplitude and lower frequency than that generated by the intact animal (Wilson, 1961). In the locust kick motor programme, however, we have no evidence for such a role for proprioception. Proprioceptive manipulation can strongly affect the probability of the occurrence of co-activation, but when co-activation does actually occur, the strength and duration of FETi output, which most clearly reflect the overall amplitude of the behaviour, are unchanged by proprioceptive manipulation.

A third proposed function for proprioception in rhythmic systems has been the reconfiguration of a neural network by modulation of the properties of the neuronal constituents (e.g. Hooper and Moulins, 1989, 1990). In the locust, we find that the FCO can exert an influence which has functional similarities to such a reconfiguration. The antagonist flexor and extensor motoneurones can participate in two sorts of behaviour, one of which requires alternating activation (e.g. walking, thrusting, struggling) and the other of which requires co-activation (kicking, jumping and swimming). The switch from alternate activation in the initial flexion stage to co-activation is strongly influenced by the FCO (Jellema *et al.* 1997). The FCO also modulates the effectiveness of both a central connection from FETi to the flexor motoneurones (Jellema and Heitler, 1996), and the input from proprioceptors signalling cuticle strain (Jellema and Heitler, 1997) so as to regulate them in a manner appropriate for the behaviour pattern currently being executed. We do not know the precise mechanisms for these effects in the locust, but in both cases the proprioceptive regulation allows the same neurones to participate in behaviour patterns that have widely differing coordination requirements.

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