

LEG COORDINATION IN THE STICK INSECT *CARAUSIUS MOROSUS*: EFFECTS OF CUTTING THORACIC CONNECTIVES

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Summary

Behavioral studies of stick insects have identified six mechanisms which coordinate leg stepping. All six are active between ipsilateral leg pairs. As a first step towards locating the neurons mediating these interactions, the present study describes the effects of cutting one of the paired thoracic connectives. After the operation the following changes in step coordination occurred. The ipsilateral leg immediately caudal to the severed connective generally showed weak stepping. In free-walking animals it often remained near its posterior extreme position and dragged along the substratum. During supported walking, rhythmic stepping was common, but the swing phase of this leg was longer and both temporal and spatial coordination were disturbed. When the leg made a pause it usually stopped in the air near the end of its swing movement. During steady walking, the operation interrupted information from the adjacent forward leg normally used to guide the end-point of the swing or to signal errors in leg placement and elicit a corrective treading-on-tarsus reflex. It also interrupted position information affecting the start of the swing. For the leg rostral to the cut, the inhibition during the swing of the posterior leg and the excitation when the latter started its retraction were both interrupted. These results indicate that all six ipsilateral coordination mechanisms are primarily mediated by the ipsilateral connective. In addition, the data show that contralateral coordination within the segmental ganglion is strongest for the front legs, weaker for the rear legs, and not discernible for the middle legs.

Introduction

Numerous studies of insect walking have led to the hypothesis that each leg is controlled by its own local center and that coordination of the legs is governed both by direct neural influences transmitted from one local center to another and by indirect influences arising from the mechanical coupling of the legs (for reviews see Delcomyn, 1985; Graham, 1985; Bässler, 1986).

The most detailed investigations of these interleg coordination mechanisms have been carried out on the stick insect. In this insect six different, neurally

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mediated interactions have been identified; these act between adjacent ipsilateral legs or between the two legs of a segment (Fig. 1). No direct interactions between more distant legs have been found. After middle leg amputation, ipsilateral front and rear legs remain coordinated and adopt a new phase (Wendler, 1964). This coordination has been proposed to be due to direct interaction (Wendler, 1968) but it may be mediated through the activity of mesothoracic circuits (which is evident in the movement of the middle-leg stump). In some arthropods diagonal leg pairs, e.g. left front leg and right middle leg, appear to step simultaneously, suggesting a direct coupling (von Holst, 1935; Graham, 1972). Such a mechanism has been included in several models (Cruse, 1979, 1980), but no direct interactions between diagonal leg pairs have been demonstrated.

The pattern of coordinating mechanisms (Fig. 1) closely resembles the structure of the insect nervous system – segmental ganglia with paired longitudinal connectives joining each thoracic hemiganglion with the next anterior and

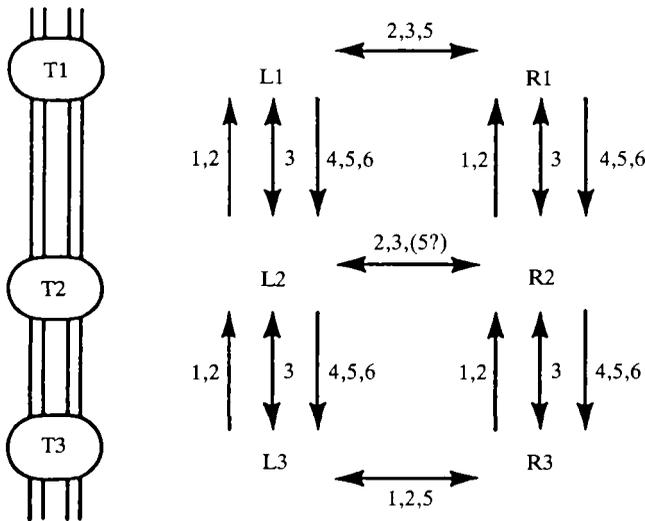


Fig. 1. Schematic diagram of coordination mechanisms in the stick insect. Legs are denoted as left (L) or right (R) and numbered from front to back. The arrows indicate the direction in which the control acts. The numbers identify the following mechanisms: (1) during its swing, the controlling leg inhibits the controlled leg from beginning a swing (Dean & Wendler, 1982; Cruse & Epstein, 1982), (2) for a brief time following the start of active retraction, the controlling leg excites the controlled leg to begin a swing (ipsilateral, Cruse & Schwarze, 1988, contralateral, Cruse & Knauth, 1989), (3) an increased resistance to retraction of the controlling leg increases the force exerted by the controlled leg (Bässler, 1979; Cruse, 1985c), (4) the position of the controlling leg influences the swing end-point (targeting behavior; Cruse, 1979; Dean & Wendler, 1983), (5) for more posterior positions the controlled leg excites a swing (Dean & Wendler, 1983; ipsilateral, Cruse & Schwarze, 1988; contralateral, Cruse & Knauth, 1988), (6) stimulation of the controlling leg occurring as the controlled leg is near its anterior extreme position (AEP) causes the controlled leg to shorten its swing or to lift off and step farther to the rear (treading-on-tarsus reflex; Graham, 1979b).

posterior hemiganglia. The present paper focuses on whether the ipsilateral influences are transmitted in the ipsilateral connective, by examining the effect of cutting the connectives.

The effect of severing thoracic connectives has not previously been studied in the stick insect, but the experiment has been performed in several other insects, most often in cockroaches. Severing one connective in free-walking cockroaches (*Blatta orientalis*) has been found to induce only slight timing differences, whereas cutting both connectives in one segment eliminated the coordination across the cut (Hughes, 1957). The latter experiment had previously been performed by several investigators on a variety of insects to test whether mechanical influences alone suffice to maintain coordination, generally with negative results (see Hughes, 1957). Cutting a single thoracic connective in tethered American cockroaches walking on an air-supported ball results in widespread changes in coordination which are most prominent in the leg pair adjacent to the cut (Greene & Spirito, 1979). Using physiological methods in combination with partial connective lesions, Pearson & Iles (1973) identified intersegmental neurons in the ipsilateral connective which could mediate the alternating activity in adjacent ipsilateral legs during righting responses.

Materials and methods

The animals used were adult female stick insects, *Carausius morosus*, from the Bielefeld laboratory stock. To sever a connective, a small flap was cut in the ventral thoracic cuticle approximately midway between the segmental ganglia. The paired connectives were exposed and one was severed using iridectomy scissors. Finally, the cuticle flap was closed and glued in place, sometimes with the aid of a small piece of tissue paper to cover the incision. Operations were performed on 10 animals, several of which were tested on more than one occasion. Walks were recorded before the operation and then at times ranging from 30 min to several months after the operation. Two animals examined at the end of the experiments showed no obvious regrowth of fibers between the cut ends of the connective. The data presented here are from five animals (eight test sessions) in which one connective between pro- and mesothoracic legs was severed and five animals (13 test sessions) in which one connective between meso- and meta-thoracic legs was severed. Six animals underwent a second operation in which the remaining ipsilateral thoracic connective was cut.

Step coordination was recorded using previously described methods (Dean, 1984). Each animal was glued dorsally to a holder which was fixed in place over a light, Styropor walking wheel (radius 20 cm, rim width 1.0 cm, moment of inertia equivalent to about 3.4 g applied at the rim). The body was thus fixed in space and the animal walked by turning the wheel underneath it. Leg movement was registered with a video camera connected to an Apple computer (Godden & Graham, 1983). This system detects the point at which each leg intersects a scan line parallel to the long axis of the body and about 3 mm lateral to the subcoxal

joint. The anterior/posterior position of this intersection is approximately proportional to the anterior/posterior position of the tarsus. The resolution of the system (1 video unit) is about 0.35 mm along the sample line. The system records data in segments about 25 s long. These were plotted and the transitions from stance to swing and swing to stance were measured from the plots using an Apple digitizing tablet. These values were stored and used to calculate statistics and histograms for position, time intervals and phase relationships.

Mean values for positions and time intervals were compared using the Behrens/Fisher *t*-test. Mean phase values and concentration parameters were calculated using circular statistics (Batschelet, 1965). The circular distributions of phase values were unimodal, so the presence of a non-uniform distribution and a preferred phase was determined using the Rayleigh test. Differences between mean phase angles were compared using the Watson/Williams procedure (Batschelet, 1965).

Results

An intact adult stick insect walking on the treadwheel showed an even, metachronal coordination (Fig. 2): the duration of the swing was nearly constant and independent of walking speed and the steps of the three ipsilateral legs

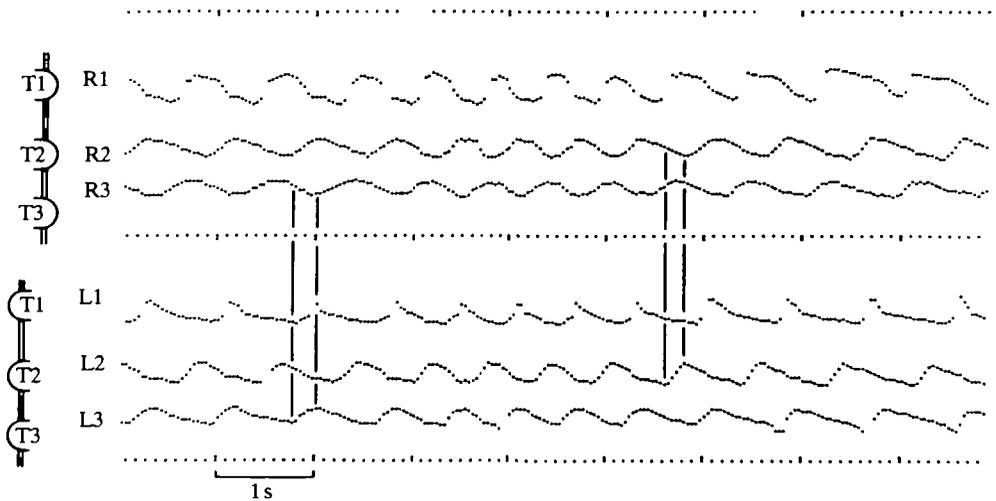


Fig. 2. Step coordination of an intact animal. The diagram shows results from the video system, which records femur positions of the six legs. Upward change in a trace corresponds to forward movement of the leg. The legs are labeled as left or right and numbered from front to back. The schematic drawings on the left of this and subsequent figures show the thoracic ganglia and indicate which connectives are intact. The two sets of vertical lines illustrate normal timing relationships: e.g. the swing phase of the left rear leg (L3) occurs while the adjacent middle leg (L2) and the contralateral rear leg (R3) are in stance.

appeared to be organized in a wave running from back to front. Although the animal was supported by the holder, the pattern retained the general feature that adjacent legs did not swing at the same time but alternated so that the swing of each leg occurred during the stance of the adjacent legs.

Effects of cutting a connective between the middle and rear legs

Severing a single connective between the mesothoracic and the metathoracic ganglion significantly disrupted the coordination of the ipsilateral middle and rear legs. The most obvious effect in unsupported animals walking on a smooth surface was an apparent weakness in the rear leg, an inability to make a strong swing movement. The ipsilateral front and middle legs continued to step in alternation with their segmental partners but the ipsilateral rear leg spent much of its time extended near its posterior extreme position (PEP) where it dragged along the surface. This leg contributed support to the animal, as was evident when the ipsilateral middle leg or the contralateral rear leg made a swing, but it seemed to lack the excitation needed to lift free of the substratum and swing forward. Only when it was unloaded by the other legs could it occasionally make a short swing.

When the animal walked on the treadmill, the need for adequate support was removed and the rear leg was able to make regular swing movements. Under these conditions the rear leg occasionally remained in the air in an anterior position during several steps of the other legs. More often it continued to step in approximately 1:1 rhythm with the other legs (Fig. 3A), but the swing movements were slower and they were poorly coordinated with those of the other legs. In general, the animals tended to walk more slowly after the operation (Table 1).

Quantitative analysis revealed differences in both step timing and leg placement. The differences in placement were generally small. Under the conditions studied here the shifts in the anterior and posterior extreme positions (AEP and PEP; Bässler, 1972) of the ipsilateral rear leg were somewhat variable. There was a tendency for the AEP and PEP of both rear legs to be more to the rear. The ipsilateral rear leg showed the largest shifts (Table 2: three of the five animals for both AEP and PEP). Some of the shifts observed in individual animals were as large as 15 video units (for comparison, step amplitudes in intact animals ranged from 16 to 23 video units) but, owing to the large number of steps in individual samples, differences as small as 1.5 units were often highly significant statistically – a fact noted by several authors (Greene & Spirito, 1979; Dean & Wendler, 1983; Delcomyn & Cocatre-Zilgien, 1988). As a result, individual animals or even different test sessions for one animal showed significant shifts but the direction was not always consistent. For the ipsilateral rear leg, the weighted mean for all animals showed caudal shifts (with respect to the values measured before the operation) of 6.0 units for AEP and 4.9 units for PEP; for the contralateral rear leg there was a shift of 1.6 units for AEP and 1.9 units for PEP. More consistently observed was an increase in the variance of these values: for the ipsilateral rear leg this was observed in 12 of 13 test sessions for the PEP and in all test sessions for the AEP or for all five animals overall (Table 2). For the ipsilateral rear leg the

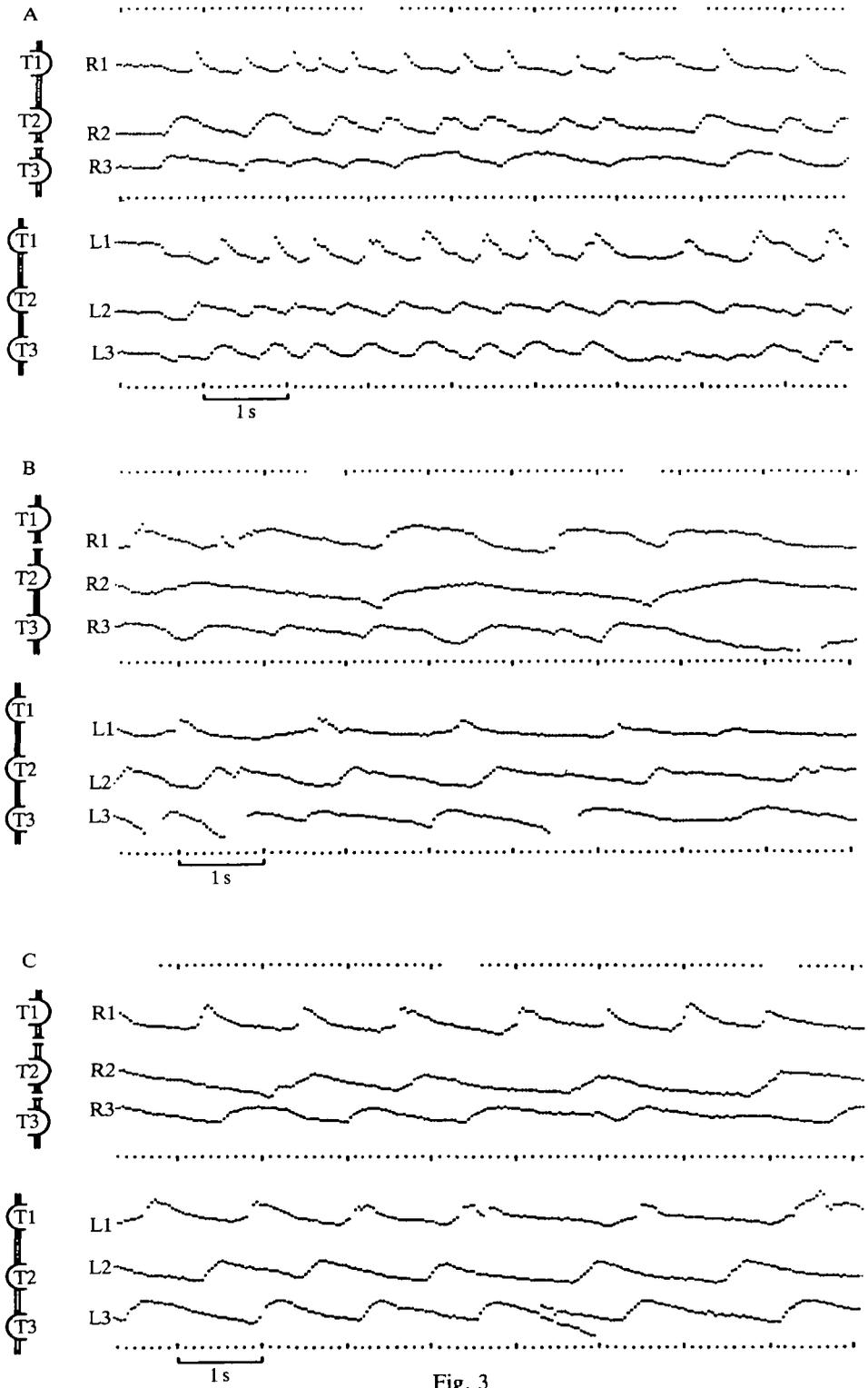


Fig. 3

Fig. 3. Effects of cutting thoracic connectives. The format is the same as that of Fig. 2. The operations are as follows: (A) right metathoracic connective, (B) right mesothoracic connective, and (C) right metathoracic and mesothoracic connectives. (The double trace in one L3 step in C is an infrequent artefact occurring when the system also registers the tibia – lower set of points – for part of the retraction.)

standard deviations of the overall means increased by 114 % for AEP and 67 % for PEP, whereas for the contralateral leg they increased by only 62 % for AEP and 30 % for PEP.

The PEP of the ipsilateral middle leg was also investigated. Following the operation, the weighted mean PEP for the ipsilateral middle leg showed no change, whereas that of the contralateral middle leg was slightly rostral to the mean observed before the operation. Individual changes were about evenly distributed. On average, the standard deviations for both legs increased over values for the intact condition by 17 % ipsilaterally and 32 % contralaterally.

Although these spatial changes were small, they occasionally had major consequences. The increase in spatial variability sometimes caused the ipsilateral rear leg to step past or onto the ipsilateral middle leg instead of immediately caudal to it. When this overstepping occurred, the rear leg often held the middle leg fast and obstructed the latter in its attempt to begin a swing. Thus, there was no sign of the treading-on-tarsus reflex (TOT reflex; Graham, 1979*b*) which normally corrects such placement errors by inducing the rear leg to lift again and step more to the rear.

Temporal coordination showed more striking changes following the operation. Again these were most evident in the ipsilateral rear leg. One of the most conspicuous features of walking in the intact animal is that the swing of a middle leg usually begins immediately after, but almost never before, the end of the swing of the ipsilateral rear leg (Figs 2, 4A). This was no longer true for the middle and rear legs of animals in which the ipsilateral connective between the two had been severed: the end of a swing by the rear leg was more or less symmetrically distributed around the start of the middle leg's swing (Fig. 4A). Small positive intervals in this histogram represent occasions when the middle leg lifts from the wheel before the rear leg completes its swing; such overlapping swings did not occur on the intact side.

Overlapping swing movements could result from an increase in the duration of the rear leg's swing; such an increase was actually observed in four of the five animals (Table 1: the smaller increase in the swing duration of the contralateral rear leg did not lead to overlapping swings). However, it also reflected a change in when the swing began: the start of the rear leg's swing was much less well coordinated with the middle leg after the operation (Fig. 5A, Table 3). In six of 13 test sessions the phase relationship was random; the range of concentration parameters for individual test sessions after the operation was 0.04–0.40 as against 0.69–0.78 for the same animals before the operation. Where a significant phase preference existed (four of five animals) and differed significantly from the intact

Table 1. Step period and swing duration, in seconds, for hind legs (HL) before and after severing one metathoracic connective

Animal	Contra HL period				Ipsi HL swing				Contra HL swing						
	Before		After		Before		After		Before		After				
	Mean	S.D.	d	N	Mean	S.D.	d	N	Mean	S.D.	d	N			
4	0.71	0.21	***	1.01	0.50	0.22	0.06	***	0.31	0.20	0.19	0.06	***	0.23	0.11
	162			320		166		336			166			342	
5	0.87	0.42	***	1.58	0.68	0.22	0.06	***	0.53	0.53	0.22	0.08	***	0.30	0.15
	105			254		105		152			107			266	
6	0.86	0.33	**	0.99	0.51	0.23	0.08	*	0.27	0.20	0.20	0.07	NS	0.20	0.12
	134			264		130		214			136			276	
7	0.86	0.29	***	1.29	0.63	0.23	0.08	NS	0.25	0.14	0.22	0.08	NS	0.22	0.11
	118			232		117		107			120			244	
10	0.61	0.19	***	1.02	0.50	0.19	0.05	***	0.34	0.36	0.18	0.05	***	0.23	0.10
	159			374		156		322			162			395	
Sum	0.76	0.28	***	1.15	0.55	0.21	0.06	***	0.33	0.3	0.19	0.06	***	0.23	0.11
	678			1444		674		1131			691			1523	

For each animal the first row shows means, standard deviations (s.d.), and the significance of differences (d) between the means before and after the operation.

Significance levels are shown as follows: $P < 0.001$ (***), < 0.01 (**), < 0.05 (*) and not significant (NS).

The second row gives the number of steps included.

The final pair of rows (sum) shows values for all animals combined.

Table 2. *Changes in spatial coordination of middle legs (ML) and hind legs (HL) as a result of severing one metathoracic connective*

Animal	Ipsi HL			Contra HL			Ipsi ML			Contra ML		
	dAEP	ds.d. (%)	dPEP	ds.d. (%)	dAEP	ds.d. (%)	dPEP	ds.d. (%)	dPEP	ds.d. (%)	dPEP	ds.d. (%)
4	8.6	***	10.9	***	2.1	***	180	NS	3.6	***	124	***
5	0.9	NS	1.3	NS	-3.1	***	118	NS	-6.7	***	104	NS
6	4.5	***	-4.6	***	4.7	***	141	***	0.2	NS	73	NS
7	14.5	***	15.6	***	5.7	***	193	***	7.0	***	130	***
10	0.6	NS	1.6	***	-0.6	NS	210	NS	-2.2	***	171	***
Sum	6.0	***	4.9	***	1.6	***	162	1.9	0.4	NS	117	***

Shifts in anterior extreme position (AEP) and posterior extreme position (PEP) are presented as differences (d) between post- and pre-operation values measured in video units; positive differences correspond to caudal shifts.
 Changes in standard deviations are given as ratios of post- to pre-operation values as a percentage.
 For intact animals, typical step amplitudes measured in video units are 16-23; typical standard deviations are 4-6 for the rear leg PEP and front leg AEP (owing to the more acute angle formed between the leg and the sampling line) and 2-3 for other position values.
 The significance of position differences is indicated for $P < 0.001$ (***), < 0.01 (**), < 0.05 (*) and not significant (NS).

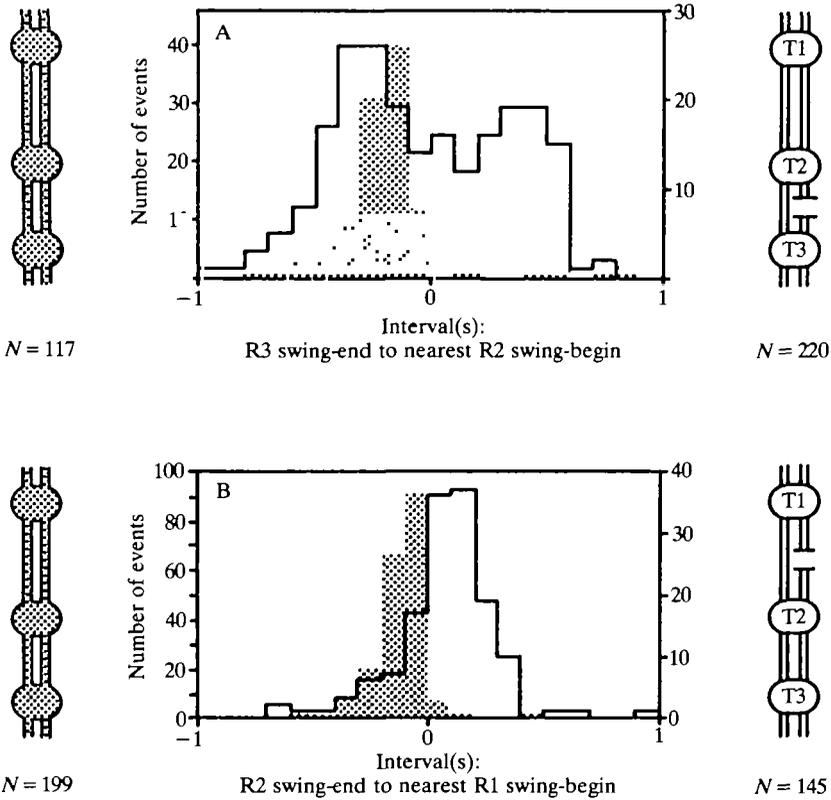


Fig. 4. End of the caudal leg's swing in relation to the beginning of the next rostral leg's swing. The figure compares for one animal the timing before and after severing one connective in the metathorax (A) or mesothorax (B). The histograms show the interval to the end of a leg's swing from the nearest start of a swing by the next rostral leg. In the intact animal (shaded histograms), the former closely precedes the latter; after the operation (outlined histograms), the former frequently follows the latter.

value (two of four animals), the rear leg stepped earlier in the cycle of the middle leg. The residual coordination was weaker than that of the contralateral middle leg/rear leg pair in 11 of 13 sessions or in all five animals overall. On the intact side the concentration parameters in individual sessions ranged from 0.24 to 0.68 after the operation; all but one were significant. A significant phase increase of 20–50° was found in three animals, a decrease of 28° in one and no change in the fifth animal.

In intact animals, the intrasegmental coordination was usually poorest between the two rear legs. When one rear leg was deprived of the ipsilateral connection to the mesothoracic ganglion the contralateral coordination was even poorer (Table 3). Concentration parameters ranged from 0.07 to 0.34 as against 0.43–0.69 for the intact tests. In two of five animals this contralateral coordination was no longer significant. The remaining three animals showed significant phase changes

in different directions. Relative to the cycle of the leg contralateral to the operation the ipsilateral leg stepped earlier in two animals (e.g. Fig. 5B) and later in the third. The middle legs remained significantly coordinated; concentration parameters ranged from 0.23 to 0.44 as against 0.58–0.76 for control sequences. There was no consistent phase shift, although three animals showed significant changes. The range of average phases was 162–214° for controls and 161–218° for operated animals.

The operation also affected the coordination of leg pairs more distant from the operation site. For both ipsilateral and contralateral sides the preferred phase of the middle leg to the front leg shifted to slightly later phases (median of 183° and

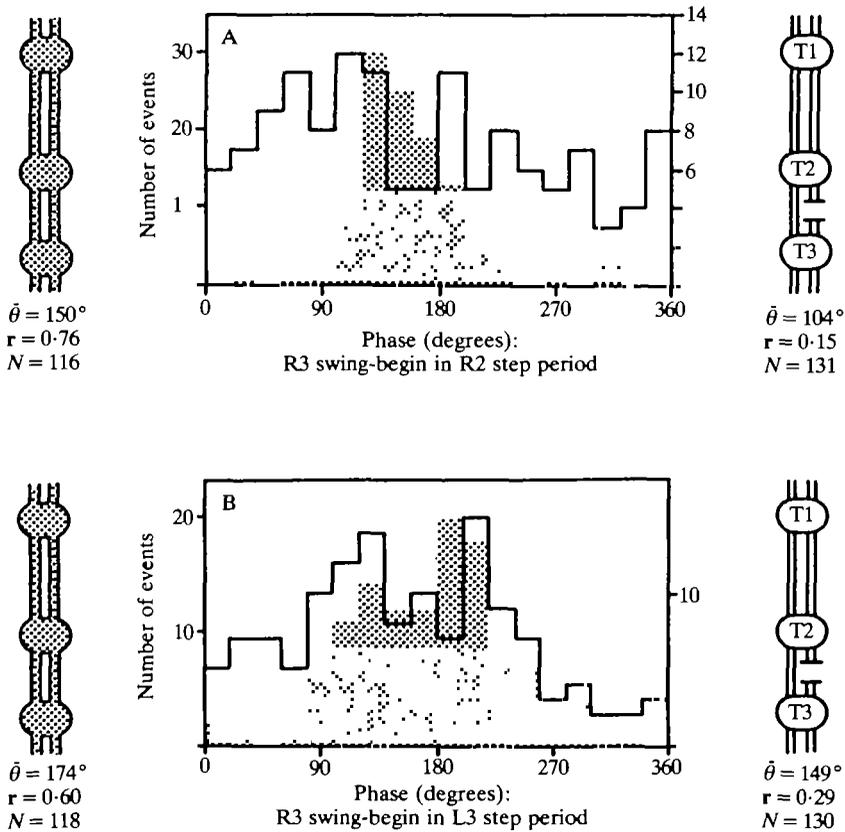


Fig. 5. Phase relationships of the ipsilateral rear leg before and after cutting one metathoracic connective. The histograms show the phase with which the rear leg's swing begins in the step cycle of the middle leg (A) and in that of the contralateral rear leg (B). In A the beginning of the rear leg's swing is shifted to earlier phases and the coupling between the legs is much weaker. In B the beginning of the ipsilateral rear leg's swing is shifted to earlier phases and the strength of the contralateral coupling is decreased. The values of the mean phase ($\bar{\theta}$), the length of the mean vector (r), and the number of steps (N) are shown in the lower left and right corners for the pre- and post-operation tests, respectively.

Table 3. Phase relationships ($\bar{\theta}$) and concentration parameters (r) for middle legs (ML) and hind legs (HL) before and after cutting one metathoracic connective

Animal	Ipsi HL in ML						Contra HL in ML						HL in contra HL						ML in contra ML					
	Before		After		Before		After		Before		After		Before		After		Before		After		Before		After	
	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	$d\bar{\theta}$	$\bar{\theta}$	r	
4	158.2	0.72	NS	169.8	0.13		199.2	0.82	***	171.7	0.54	163.4	0.43	***	210.0	0.16	214.0	0.76	NS	217.6	0.33			
5	168.1	0.69	NS	171.2	0.17		189.4	0.67	***	231.4	0.44	169.6	0.44	**	131.0	0.24	194.7	0.63	***	161.3	0.36			
6	195.2	0.69	***	99.9	0.2		133.4	0.52	***	183.8	0.33	241.5	0.55	-	194.4	0.07	NS	171.8	0.57	NS	166.0	0.23		
7	150.5	0.76	-	87.5	0.12	NS	158.4	0.73	***	180.0	0.53	174.0	0.60	**	142.8	0.28	176.8	0.58	***	211.0	0.42			
10	160.0	0.78	*	128.6	0.12		164.3	0.80	NS	168.8	0.48	157.7	0.69	-	172.5	0.04	NS	161.8	0.79	***	192.1	0.32		
Sum	165.2	0.71	***	134.6	0.12		172.2	0.66	**	182.6	0.44	177.3	0.47	NS	165.2	0.1	184.3	0.63	•	193.8	0.31			

Differences (d) between phases ($\bar{\theta}$) before and after the operation are indicated for $P < 0.001$ (***), < 0.01 (**), < 0.05 (*) and not significant (NS).

Concentration parameters (r) which do not reach significance at the 0.05 level are marked (NS); because the corresponding phase values cannot be assigned a confidence interval the differences cannot be tested (-).

The overall mean phase and concentration parameters are calculated using circular statistics.

range of 130–205° for the pre-operation tests as against a median of 217° and range of 183–265° after the operation) but the coupling remained fairly normal (median of 0.69 and range of 0.32–0.81 as against a median of 0.59 and range of 0.26–0.83). Inspection of lag–step period relationships indicated that these small but consistent phase shifts arose from the increase in step period (Fig. 6). On the ipsilateral side this coordination of front and middle legs meant that front and rear legs also

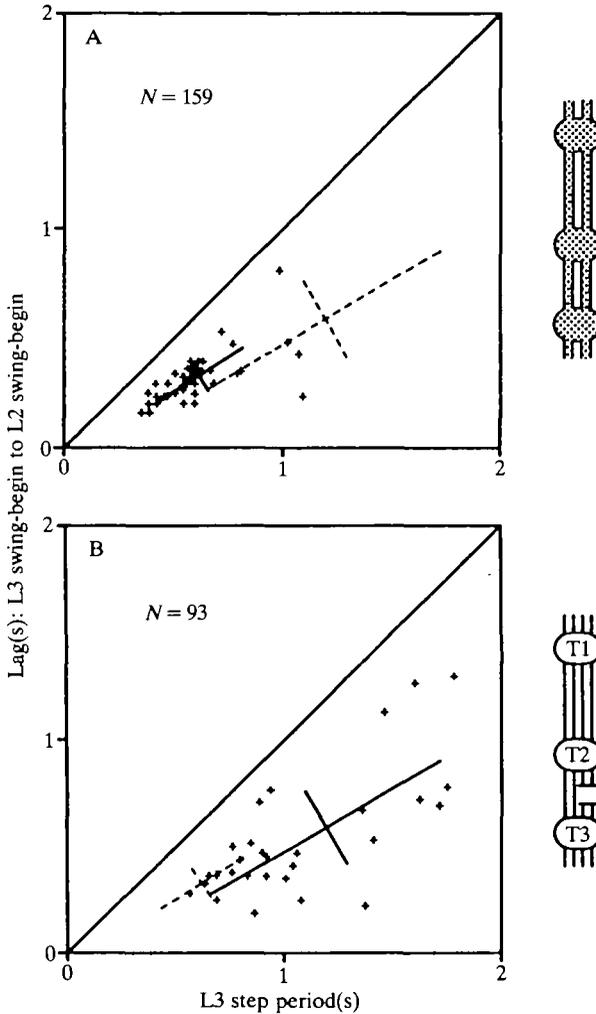


Fig. 6. Comparison of lag intervals on the unoperated side before and after severing the right metathoracic connective. The figure shows a plot of the lag from the beginning of the left rear leg's swing to the beginning of the left middle leg's swing as a function of step period before (A) and after (B) the operation on the right connective. By definition, the lag cannot be greater than the period, so all points lie below the diagonal. The crosses show the principal axes of the distribution; to aid comparison, each cross is also shown as dashed lines in the other part of the figure. To limit overlap of points, only every fifth step is plotted; in B one outlier has been omitted.

showed some coordination. In the five sessions in which a significant phase preference remained, the ipsilateral front and rear legs continued to step in phase. The contralateral coordination between the two front legs weakened in three animals (median of 0.60 and range of 0.33–0.82 for the intact condition as against a median of 0.53 and range of 0.29–0.66), but only one animal showed a significant phase shift.

Effects of cutting a connective between the front and middle legs

Severing a connective between the prothoracic and mesothoracic ganglia led to changes in the stepping of the ipsilateral middle leg analogous to those described above for the rear leg. During unsupported walking the ipsilateral middle leg usually remained in a posterior position, where it was dragged over the ground. Because the ipsilateral front and rear legs together provide sufficient support for the animal during their common stance, the middle leg was sometimes able to make long, slow swing movements. During supported walking the ipsilateral middle leg often remained in the air in an anterior position but long segments of 1:1 stepping were also common (Fig. 3B).

The pattern of spatial changes was complex. The mean AEP and PEP of the ipsilateral leg caudal to the operation, in this case the middle leg, showed little change (Table 4), but their standard deviations increased (five of eight test sessions for AEP and eight of eight for PEP). Following the operation, the standard deviations of the overall means increased by 79 % for AEP and 198 % for PEP. For the contralateral middle leg, the corresponding standard deviations increased only slightly (22 % for AEP and 37 % for PEP), but the mean positions shifted caudally by 1.5 units for AEP and 2.1 units for PEP. The ipsilateral front leg differed from the other legs in showing a rostral shift of the PEP in four of five animals, whereas that of the contralateral front leg shifted caudally in all sessions; expressed as a weighted mean over all animals, the PEP of the ipsilateral front leg shifted 3.1 units rostrally while that of the contralateral leg shifted 3.5 units caudally. The standard deviation of the PEP increased for ipsilateral front legs (110 %) and contralateral front legs (65 %). For both rear legs, the mean AEP and PEP shifted caudally. The ipsilateral rear leg showed larger changes; caudal shifts occurred in all animals and the weighted means for AEP and PEP changed by 7.5 units for AEP and 9.6 units for PEP. The standard deviations of the AEP increased in all animals; that of the PEP increased in six of eight test sessions. The increases in the standard deviations of the overall means were 116 % for AEP and 76 % for PEP. The contralateral rear leg showed smaller changes in position and variability. Both AEP and PEP were more to the rear in six of eight sessions; the weighted mean positions changed by 2.2 units for AEP and 5.4 units for PEP. The standard deviations also increased in six of eight sessions; the overall standard deviations increased by 120 % for AEP and 45 % for PEP.

The decline in the spatial coordination of the ipsilateral middle and front legs was evident in that the middle leg often stepped onto the tibia or femur of the front

Table 4. Shift in AEP and PEP and change in standard deviation as a result of severing one mesothoracic connective

Animal	Ipsi ML			Contra ML			Ipsi FL			Contra FL			Ipsi HL			Contra HL			
	dAEP (%)	dPEP (%)	ds.d. (%)	dAEP (%)	dPEP (%)	ds.d. (%)	dAEP (%)	dPEP (%)	ds.d. (%)	dAEP (%)	dPEP (%)	ds.d. (%)	dAEP (%)	dPEP (%)	ds.d. (%)	dAEP (%)	dPEP (%)	ds.d. (%)	
1	-2.9 ***	184 -3.7 ***	223	1.8 ***	89	0.5 NS	156	-5.7 ***	200	4.2 ***	104	6.8 ***	173	12.7 ***	144	-1.1 ***	122	0.7 NS	104
2	-0.3 NS	222 3.2 **	296	0.5 NS	129	3.1 ***	154	-5.9 ***	224	2.6 ***	167	7.8 ***	162	10.3 ***	292	4.6 ***	250	8.9 ***	163
3	2.7 ***	236 2.3 NS	508	1.5 ***	75	0.3 NS	79	-3.3 ***	210	2.0 ***	132	14.3 ***	196	17.4 ***	219	1.5 ***	154	4.2 ***	128
8	7.3 ***	112 -1.8 **	289	1.5 **	193	1.8 ***	131	-2.1 **	195	3.5 ***	169	3.8 NS	235	1.1 NS	127	1.8 ***	141	4.5 ***	130
9	-0.4 NS	224 1.4 NS	269	1.8 ***	162	4.8 ***	144	1.7 ***	196	4.4 ***	177	5.4 ***	329	5.8 ***	169	4.7 ***	251	9.3 ***	156
Sum	0.7 *	179 -0.3 NS	298	1.5 ***	120	2.1 ***	137	-3.1 ***	210	3.5 ***	165	7.5 ***	216	9.6 ***	176	2.2 ***	220	5.4 ***	145

The format is the same as that of Table 2.

Table 5. Phase relationships ($\bar{\theta}$) and concentration parameters (r) before and after cutting one mesothoracic connective

Animal	Ipsi ML in FL			Contra ML in FL			ML in contra ML			FL in contra FL			HL in contra HL		
	Before $\bar{\theta}$	After $\bar{\theta}$	r	Before $\bar{\theta}$	After $\bar{\theta}$	r	Before $\bar{\theta}$	After $\bar{\theta}$	r	Before $\bar{\theta}$	After $\bar{\theta}$	r	Before $\bar{\theta}$	After $\bar{\theta}$	r
1	160.8 0.71	*** 245.1 0.31	175.8 0.76	*** 219.0 0.74	158.9 0.70	*** 208.9 0.31	171.3 0.71	** 187.0 0.67	160.6 0.53	NS 169.3 0.35					
2	190.9 0.83	NS 201.8 0.24	178.5 0.76	*** 222.8 0.78	179.9 0.82	- 127.1 0.14	NS 168.0 0.81	** 200.8 0.54	176.8 0.78	* 149.4 0.33					
3	148.4 0.72	- 99.6 0.15	NS 211.8 0.69	** 190.0 0.68	124.1 0.58	- 241.0 0.19	NS 186.5 0.82	*** 213.1 0.65	145.9 0.70	NS 130.4 0.48					
8	175.0 0.83	NS 179.8 0.32	185.9 0.81	*** 224.6 0.72	173.6 0.82	NS 168.3 0.38	180.2 0.88	NS 192.7 0.70	156.9 0.79	NS 176.0 0.28					
9	178.0 0.82	*** 106.6 0.15	184.8 0.80	NS 194.4 0.46	147.6 0.72	NS 164.4 0.26	163.3 0.87	*** 194.7 0.60	155.2 0.70	NS 168.5 0.20					
Sum	172.1 0.76	** 192.8 0.13	184.4 0.75	*** 209.3 0.63	161.5 0.70	*** 183.4 0.24	173.0 0.80	*** 196.5 0.63	161.1 0.68	NS 161.4 0.30					

The format is the same as that of Table 3.

Fig. 7. Phase relationships of the ipsilateral middle leg before and after cutting one mesothoracic connective. The histograms show the phase with which the middle leg's swing begins in the step cycle of the front leg (A) and in that of the contralateral middle leg (B) as well as the phase of the front leg in the step cycle of the contralateral front leg (C). In this animal the beginning of the middle leg's swing is shifted to later phases and the coupling between the legs is much weaker (A), the beginning of the ipsilateral middle leg's swing is shifted to later phases and the strength of the contralateral coupling is decreased (B), but the coordination of the front legs (C) is left unchanged. See Fig. 5 for abbreviations.

leg. It then held the front leg fast and obstructed its attempts to swing forward. These errors in placement were not corrected by a TOT reflex.

The temporal coordination of the ipsilateral front and middle legs was also severely impaired. The front leg often began its swing movement before the middle leg had completed its swing (Fig. 4B). The beginning of the middle leg's swing was random or only weakly coordinated within the step cycle of the front leg (Fig. 7A, Table 5); changes in preferred phase were highly variable. The coordination of the contralateral middle and front legs remained at normal strength in four of five animals. In these animals the significant changes in mean phase which did occur varied in direction (median 185° , range $176\text{--}212^\circ$ for the intact tests compared with median 219° , range $190\text{--}225^\circ$ after the operation). Inspection of regressions of lag on step period for the contralateral leg pair indicated that these timing changes were related to changes in step period.

The contralateral coordination of the two middle legs was also weaker after the operation (Fig. 7B), whereas the two front legs continued to show good alternation (Fig. 7C).

The stepping of legs not directly adjacent to the severed connective was also affected. The swing duration of the ipsilateral rear leg was slightly lengthened but less so than that of the middle leg (Table 6). The coordination of the ipsilateral middle and rear legs weakened and in two animals was no longer significant (range of concentration parameters $0.08\text{--}0.45$); there were no significant phase shifts. The coordination of the contralateral middle and rear legs also weakened but it remained significant in all five animals (range $0.48\text{--}0.74$); the phase of the middle leg in the rear leg cycle decreased in four of these animals, reflecting increases in step period. The coordination of the ipsilateral rear leg in the step cycle of the front leg generally remained significant (five of seven test sessions) and in these cases the two legs continued to alternate. The contralateral coordination of the two rear legs became weaker but remained significant in all five animals; a significant phase change – towards earlier phases – was present in only one animal.

Effects of cutting both thoracic connectives on one side

The combined operation (six animals) generally led to very weak stepping by the ipsilateral middle and rear legs (Fig. 3C). Protraction movements were often very long, lasting through many steps of the other legs. The rear leg was more likely than the middle leg to show normal stepping.

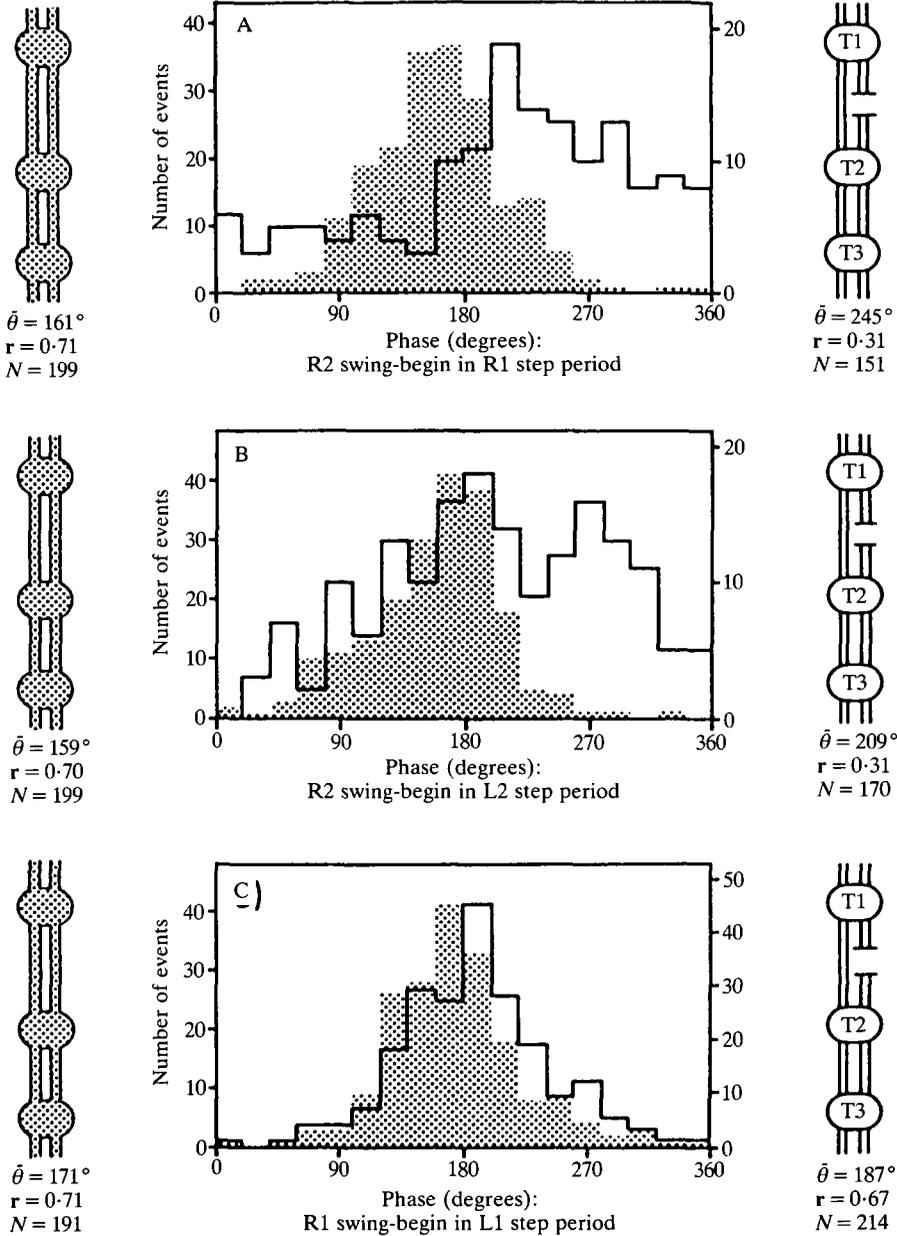


Fig. 7

Although the number of steps that could be collected was often small, the results qualitatively support those from the single operations. The ipsilateral coordination among front, middle and rear legs was decidedly poorer. Both front and middle legs often began a swing while the adjacent posterior leg was still in the air. Similarly, the coordination of the start of a swing within the step cycle of the

Table 6. Step period and swing duration, in seconds, for hind legs (HL) and middle legs (ML) before and after severing one mesothoracic connective

Animal	Contra ML period				Ipsi ML swing				Contra ML swing				Ipsi HL swing				Contra HL swing								
	Before		After		Before		After		Before		After		Before		After		Before		After						
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.			
1	0.53	0.16	***	0.81	0.30	0.18	0.06	***	0.56	0.46	0.16	0.04	0.16	0.04	0.16	0.05	***	0.20	0.08	0.17	0.06	NS	0.17	0.06	
	199	237		184	199		184	203	245	188	253	193	237												
2	0.59	0.21	***	1.09	0.76	0.18	0.06	***	0.39	0.17	0.18	0.04	NS	0.18	0.05	0.20	0.05	***	0.24	0.13	0.19	0.06	NS	0.19	0.07
	145	91		72	151		72	149	96	148	95	150	85												
3	1.08	0.50	***	1.55	0.60	0.30	0.10	***	1.58	2.3	0.21	0.05	***	0.27	0.08	0.27	0.09	***	0.45	0.30	0.23	0.06	***	0.30	0.13
	93	172		64	100		64	96	179	100	168	82													
8	0.74	0.20	***	1.38	0.60	0.20	0.05	***	0.41	0.21	0.18	0.04	NS	0.19	0.07	0.20	0.05	***	0.28	0.20	0.17	0.04	***	0.19	0.06
	139	110		110	140		110	142	120	137	93	128	129												
9	0.86	0.34	***	1.73	0.87	0.24	0.05	***	0.48	0.29	0.20	0.04	***	0.25	0.10	0.28	0.08	***	0.42	0.46	0.26	0.08	NS	0.28	0.18
	125	249		202	128		202	128	266	126	208	122	281												
Sum	0.71	0.27	***	1.32	0.65	0.21	0.06	***	0.59	0.79	0.18	0.03	***	0.21	0.07	0.21	0.06	***	0.32	0.28	0.19	0.06	***	0.22	0.12
	701	859		632	718		632	718	906	699	817	675	814												

The format is the same as that of Table 1.

forward leg weakened or was no longer significant. In contrast, the legs of the contralateral side remained quite normally coordinated: the more caudal leg finished its swing before the next forward leg lifted from the wheel. Phase relationships on the contralateral side were also normal, although the strength of the coupling was generally weaker than in the intact animal.

Contralateral coordination was differently affected among the three segments. The front legs continued to show good stepping and alternation. Phase relationships and concentration parameters were virtually normal (Fig. 8A, Table 7).

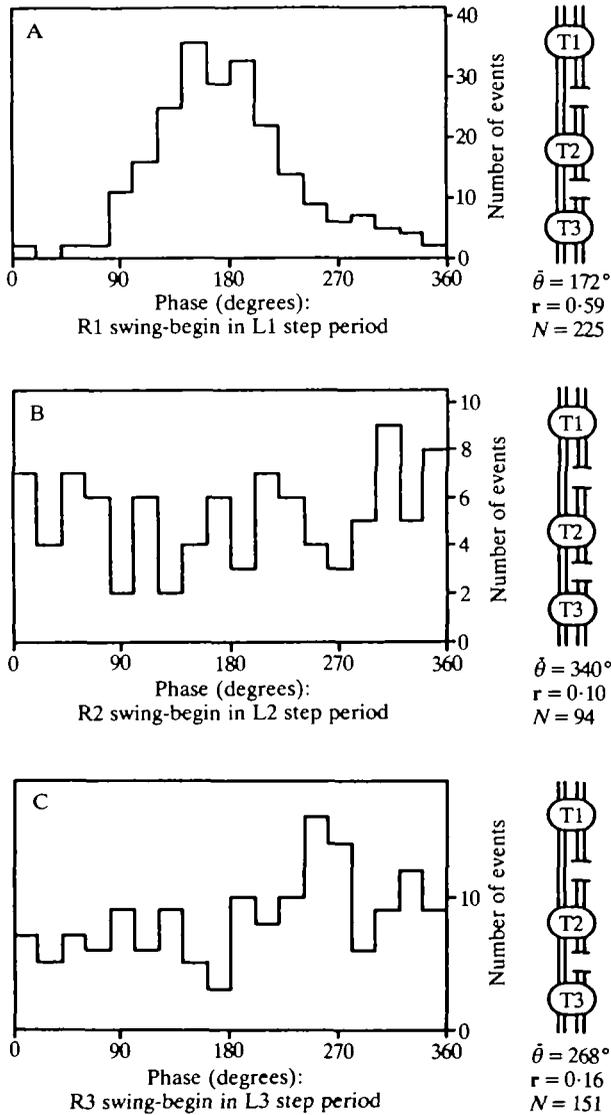


Fig. 8. Contralateral coordination of all three leg pairs after unilateral cutting of the mesothoracic and metathoracic connectives. See Fig. 5 for abbreviations.

Table 7. Phase relationships ($\bar{\theta}$) and concentration parameters (r) before and after cutting one mesothoracic and one metathoracic connective ipsilaterally

Animal	FL in contra FL				ML in contra ML				HL in contra HL				
	Before		After		Before		After		Before		After		
	$\bar{\theta}$	r	$d\bar{\theta}$	r	$\bar{\theta}$	r	$d\bar{\theta}$	r	$\bar{\theta}$	r	$d\bar{\theta}$	r	
1	171.3	0.71	**	187.1	0.71	158.9	0.70	—	160.6	0.53	NS	163.3	0.20
2	168.0	0.81	***	193.5	0.75	179.9	0.82	—	176.8	0.78	—	204.7	0.29
3	186.5	0.82	NS	179.4	0.52	124.1	0.58	—	145.9	0.70	**	194.5	0.22
7	174.0	0.60	NS	171.5	0.59	176.8	0.58	—	174.0	0.60	***	268.5	0.16
9	163.3	0.87	***	195.5	0.77	147.6	0.72	—	155.2	0.70	—	167.1	0.23
10	184.3	0.82	*	170.7	0.57	161.8	0.79	—	157.7	0.69	***	87.2	0.15
Sum	174.0	0.76	*	179.9	0.62	160.9	0.68	—	163.0	0.65	*	185.4	0.1

The format is the same as that of Table 3.

Significant phase coupling in the middle legs could not be demonstrated, either because of inadequate stepping (two animals) or because of random phase distributions (four animals, Fig. 8B). The rear legs made more steps and showed better coordination (Fig. 8C). The coupling was weak (0.15–0.29) and not significant in two animals. Changes in preferred phase were variable.

Step coordination as a function of time after the operation

For some of the animals, walks were recorded several times following the operation. Most of these were in the group in which a connective between middle and rear legs was severed. The spatial coordination showed no evidence of recovery following the operation; the animals continued to step occasionally onto the adjacent forward tarsus and the TOT reflex did not return. The swing duration remained longer than that of the intact animals. Temporal coordination was poorer. For the animal tested most often the coordination of several leg pairs, in addition to the pair immediately adjacent to the operation, was no longer significant immediately following the operation (Fig. 9). Significant phase preferences returned in subsequent days. This pattern of widespread coordination loss and apparent recovery was not generally true of other animals subjected to

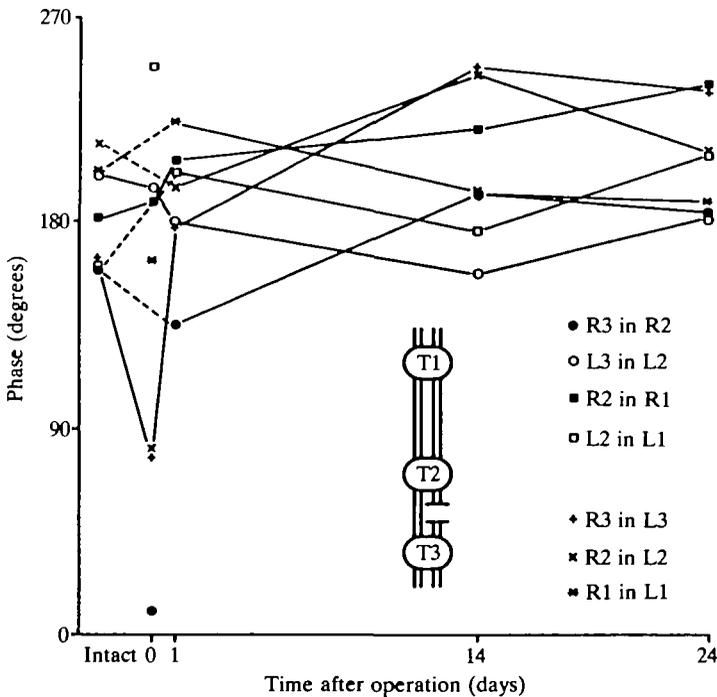


Fig. 9. Phase changes as a function of time following the operation. The figure plots the mean phase for various leg pairs, indicated by the symbols, against time after the operation. Phase angles for distributions where the concentration parameter is not significantly different from zero are plotted but not joined to the other values.

repeated measurements. The only exception involved the phase of the ipsilateral rear leg in the middle leg step cycle following a metathoracic operation: here the concentration parameters did increase with time. With respect to contralateral coupling, there was no apparent trend in preferred phase for the front or rear legs and only a slight decrease for the middle legs. In general, there was no consistent change for any of the leg pairs. The only discernible trend was a gradual increase in the phase of both middle legs in the step cycle of the ipsilateral front legs and this was generally true for all animals. This increase qualitatively accompanied increases in the step period.

Discussion

The intact stick insect walks with a step pattern that is basically regular and yet shows much variation in its details and in its adaptation to the walking situation. The occurrence of gliding coordination (Wendler, 1964) and the stepping of legs after isolation of their segmental ganglion in the stick insect and in other insects (Bässler *et al.* 1985; Hughes, 1957, and older literature cited therein; Wilson, 1966) show that each leg has its own pattern generator for rhythmic stepping.

The anatomical extent of the step generator in the stick insect is unknown. An autonomous central pattern generator has not yet been demonstrated (Bässler & Wegner, 1983), but it is certain that the movement of the leg as signalled by proprioceptors is part of the pattern generator. For various rhythmic movements of wings and legs in other insects, neuronal models have been postulated involving intersegmental elements (e.g. Robertson, 1986), but more recent results indicate that circuits within a hemiganglion are sufficient (Ronacher *et al.* 1988). The experiments reported here also indicate that intersegmental interneurons are not necessary for the generation of rhythmic stepping.

If the essential elements of the pattern generator are restricted to the segmental ganglion and possibly to the local hemiganglion, then it follows that leg coordination arises from the interaction of these individual pattern generators. This interaction may occur either directly through neural interactions or indirectly through mechanical coupling among the legs. In the free-walking insect, mechanical coupling occurs in all three spatial directions; in the supported insect walking on the treadmill the height is fixed and one important influence is removed. Mechanical coupling along the body axis, as noted above, or through limited lateral movements of the wheel continues. Changes in the force exerted by one leg alter the resistance experienced by other legs and may influence the local controller in the amount of force it applies (Cruse, 1985c) or in its decision to begin a swing (Cruse, 1985a,b). However, this information is probably insufficient to control step timing because it is unlikely that the controller for a leg can link a given change it may experience to the action of a particular leg. The decrease in coordination observed in the present experiments supports this assumption.

The direct neural interactions must involve neurons running in the connectives between the six hemiganglia of the thorax. Six different coordinating mechanisms

have been described for the stick insect (Fig. 1). These are the influences affected by the lesions studied here; they will be considered in turn.

One of these intersegmental influences affects spatial coordination. The targeting behavior (Cruse, 1979; Dean & Wendler, 1983) found in the stick insect serves to bring each leg at the end of its swing to a position slightly behind the tarsus of the leg in front of it. Thus the end position of the swing is influenced by proprioceptive information about the position of the target leg. The behavior of the operated animals indicates that this information is transmitted over the ipsilateral connective and that it serves to reduce the variance of the AEP. For both middle and rear legs, cutting the anterior connective leads to frequent errors in leg placement, such that the leg oversteps and lands on the target leg.

For the middle leg, it appears that the target information phasically adjusts the swing end-point but does not induce a net shift: the intrinsic swing end-point of the mesothoracic leg controller is approximately that shown under the influence of the targeting mechanism. For the rear leg, the targeting mechanism, or possibly other descending activity, contributes excitation for the leg to swing farther forward: after the operation, the end-points are generally more to the rear. However, the small absolute size of these shifts and the lack of consistency among animals point to a weak effect.

In the intact animal, errors in leg placement that cause one leg to step onto the adjacent forward leg are corrected by the TOT reflex (Graham, 1979*b*): the anterior leg sends the message that something has landed on it and if the existing movement of the rear leg is such that this leg might be the offender, i.e. the rear leg is forward and near the end of the swing (Schmitz & Haßfeld, 1989), then a corrective movement is triggered. Severing the ipsilateral connective apparently interrupts the signal from the forward leg, so no TOT reflex occurs. Neither the tactile stimulus for the rear leg nor the struggling of the forward leg is sufficient for the rear leg to identify its error and seek a new foothold. Thus, the information that the forward leg has been trodden upon apparently travels in the ipsilateral connective. A similar organization has been reported for the compensatory leg placement reflex, an interleg reflex in standing animals (J. Schmitz & M. Fricke, personal communication).

Information for temporal coordination also travels primarily in the ipsilateral connective. Such signals include the anteriorly directed inhibition which delays the swing of the forward leg for as long as the next caudal leg remains in swing. This influence helps ensure that swing phases in adjacent ipsilateral legs alternate (Dean & Wendler, 1982). In free-walking animals the same function is also served by mechanical coupling – when one leg is in the air during its swing, the increased load placed upon the adjacent ipsilateral legs decreases the probability that they will begin a swing (Bässler, 1977; Cruse, 1985*b*) – but this influence is removed in the current experiments. When the ipsilateral connective is severed, the rostral leg often begins its swing before the swing of the next caudal leg is completed. Thus, the inhibition is presumably conveyed through the ipsilateral connective.

In intact animals, the PEP of the forward leg may shift caudally if the duration of the inhibition is excessively prolonged (Cruse & Epstein, 1982; Cruse, 1985*b*). The forward shift in the PEP of the ipsilateral front leg when the mesothoracic connective is cut indicates that the front leg is released from an inhibition which normally delays the start of its swing slightly.

Evidence for a similar organization of intersegmental inhibition has been found in other arthropods. Hughes (1957) wrote that, after one thoracic connective had been cut, the rule 'that no leg is moved until the one behind has taken up its supporting position' is obeyed as long as the isolated leg maintains regular stepping: this alternation could represent the effects of mechanical coupling in the free-walking cockroaches. However, he also noted that the rear leg sometimes fell out of rhythm and one figure shows overlapping swing movements of rear and middle legs. The units linked by Pearson & Iles (1973) to alternation of bursting activity in the levator muscle of adjacent legs in a dissected preparation also lie in the ipsilateral connective.

The normal temporal order, in which the end of a leg's swing precedes the beginning of the adjacent rostral leg's swing, should also arise from the second anteriorly directed mechanism (mechanism 2 in Fig. 1). This mechanism tends to excite swing movements in the rostral leg shortly after the next caudal leg has initiated its retraction movement. Owing to the mechanical coupling through the wheel, which may hide a weak or purely passive retraction of the leg caudal to the severed connective, the time at which this mechanism becomes active cannot be directly determined. However, the decrease in coordination evident in the histograms indicates that this anterior excitation is also impaired by cutting the ipsilateral connective.

A similar excitation could arise indirectly. If the caudal leg makes an active retraction, then it would unload the other legs by way of the longitudinal coupling through the wheel and might trigger a swing by the forward leg in the normal step order. However, as discussed above, the mechanical coupling does not provide information specific to particular leg pairs and apparently is not sufficient to coordinate stepping (as opposed to triggering it). In free-walking insects, but not in the present situation, the normal temporal order may also be reinforced by mechanical coupling in the vertical direction: one leg may begin its swing in response to the unloading that occurs as the rear leg takes up its stance (Hughes, 1957).

A third important influence in the stick insect contributing to the alternation of ipsilateral legs is a posteriorly directed excitation. Depending upon its position and on walking speed, one leg triggers the next caudal leg to begin a swing so that the latter arrives at its AEP shortly before the forward leg reaches its PEP (Dean & Wendler, 1983; Cruse & Schwarze, 1988). When the direct connection through the ipsilateral connective is interrupted, this coordinating influence on the start of the swing shows severe deficits, particularly in the strength of coupling. When one metathoracic connective is cut, the ipsilateral rear leg tends to step earlier in the cycle of the middle leg, whereas, when one mesothoracic connective is cut, no

trend is evident and the overall change is in the opposite direction. In his study of free-walking cockroaches, Hughes (1957) also found that the leg deprived of its ipsilateral connection to neighboring legs tended to step earlier in the rhythm of the other legs, but he described the changes in coordination as slight. Greene & Spirito (1979) measured two- to threefold increases in the circular standard deviations, but the corresponding concentration parameters (0.98–0.99) remained far larger than those found for the stick insect. Unlike in the stick insect, the preferred phase for legs adjacent to the cut initially showed little change but then after 10–20 days differed considerably from pre-operation values. Following a metathoracic operation, the phase of the middle leg in the rear leg cycle generally increased; this corresponds to the decrease observed in phase, as calculated here. Following a mesothoracic operation, the phase of the front leg in the middle leg cycle generally decreased; such a change was not evident in the stick insect. In fact, if one considers the relative changes on the ipsilateral and contralateral sides, it rather appears that the preferred phase decreased so the response of the caudal leg was similar for both metathoracic and mesothoracic operations.

The residual ipsilateral coordination was poorer than the coordination of the contralateral leg pair, so there is no evidence of a direct connection between ipsilateral hemiganglia by way of the contralateral connective. Rather, it appears that the remaining coordination arises indirectly from the contralateral coordination within the segmental ganglia and the intersegmental coordination on the intact side.

Wendler (1964) observed that front and rear legs shifted from in phase to alternation following middle leg amputation and he included a direct, forward-directed influence in a later model (Wendler, 1968). The present results do not support or disprove the existence of a direct influence. Coordination is weaker after an ipsilateral connective has been severed but both this deterioration and the residual coordination may reflect chains of coupling influences through adjacent leg pairs. They do show that the indirect pathways by way of the contralateral connective generate in-phase stepping of ipsilateral front and rear legs and, therefore, the alternation observed by Wendler must have been generated by ipsilateral mechanisms.

The spatial changes in the PEP can provide information about the nature of the coupling influences. Following an operation on a metathoracic connective, the PEP of the rear leg tended to move caudally, so the influence from the middle leg apparently contributes net excitation for swing initiation. Following an operation on a mesothoracic connective, the PEP of the middle leg did not show systematic changes, so the influence from the front leg may have both excitatory and inhibitory components.

Changes in the ipsilateral coordination on the intact side have also been demonstrated. Hughes (1957) reported that the sequence remains normal but his figures suggest a slight change in timing. Pearson & Iles (1973) showed a decrease in the mean phase of the middle leg depressor activity in the cycle of the rear leg depressor following section of the contralateral connective. They considered that

indirect effects mediated by reflexes related to the altered body posture might account for these shifts. Greene & Spirito (1979) found no significant changes for tethered walking immediately after the operation, but over several weeks the mean phase of the forward leg in the caudal leg cycle declined. Since they computed the converse phase values, this decline corresponds to the gradual increase found in the present study. For the stick insect, and possibly for the cockroach since only slow walking was studied, this change can be related to changes in step period and the normal period-dependence of phase and lag.

Contralateral coordination between the legs of each segment may arise through mechanical coupling, through direct neural interactions by way of the commissures connecting the hemiganglia, or through the combined action of ipsilateral mechanisms and contralateral coupling in other ganglia. Since vertical loading is absent in the present experiments and the ipsilateral connections are interrupted by the operation, the strength of the direct contralateral coordination can be estimated. This contralateral coordination is thought to depend upon mechanisms analogous to the two excitatory ipsilateral influences (Cruse *et al.* 1986; Cruse & Knauth, 1989: mechanisms 2 and 5 in Fig. 1). In some ganglia an inhibitory influence (mechanism 1) that prevents simultaneous swings may also exist (Dean & Wendler, 1982). The present results indicate that coordination is strong between the two front legs, weak between the two rear legs and not evident between the two middle legs. In intact animals, in contrast, the middle leg coordination usually lies between that of the front and the rear legs. Whether these differences in observed coordination correspond to the actual strength of the direct coupling is hard to assess. Changes in general activation, discussed below, may reduce the apparent coordination.

These deficits in contralateral coordination did not show any systematic improvement in any of the animals tested repeatedly after the operation. In cockroaches, Greene & Spirito (1979) found changes in the contralateral coordination of segmental pairs which did vary with time. For leg pairs posterior to the lesion, the mean phase of the leg on the intact side with respect to that on the operated side increased following the operation, then gradually decreased below pre-operation values. In the stick insect, no clear change in the preferred phase could be determined. In *Periplaneta*, the leg pair immediately anterior to the lesion showed an initial change which was smaller and in the opposite direction, then the values returned to normal. Hughes's (1957) fig. 10 shows a similar initial change for *Blatta orientalis*. This change corresponds to the slight increase in phase evident in the present results for middle and front leg pairs (Tables 3 and 5, respectively) but the magnitude of this change in the middle legs was much smaller in the stick insect.

The variability between sessions for one animal and between animals prevents exact interpretations of these changes over time and of some of the spatial changes described above. As several authors have noted (Greene & Spirito, 1979; Delcomyn & Cocatre-Zilgien, 1988), even small differences may be highly significant owing to the large number of steps in the individual samples. A more

exact analysis requires a better description of the variability within and among individuals.

In addition to the interactions among the subsystems, the entire walking system is under the control of higher-level networks which turn walking on and off or influence the speed and direction of walking. Although stimuli applied to any part of the body can elicit walking, stimulation and lesion experiments have shown that much of the control and activation ultimately descends from the brain and the subesophageal ganglion (Graham, 1979a, 1985; Hughes, 1957). In cockroaches, bilateral section of the connectives in the metathorax leaves the rear legs generally inactive (Hughes, 1957). Pearson & Iles (1973) obtained a similar result by cutting only the lateral halves of both metathoracic connectives.

Bässler *et al.* (1985) reported that, following bilateral section of the metathoracic connectives in the stick insect, the rear legs tend to walk backwards when the animal is placed on a slippery surface. This tendency was not noted here. Possibly a single intact connective is sufficient to induce forward walking. The primary experimental situation is not well suited to reveal this effect: the mechanical coupling through the wheel may impose forward walking on the rear legs. However, preliminary observations of operated animals on a slippery surface indicate that when the animal is stimulated to walk forward then the ipsilateral rear or middle legs also walk forward.

In the current experiments, changes in the general activation of walking would primarily affect swing movements, since the mechanical coupling of the legs during stance would conceal small changes in retraction force. Initial tests with supported animals on a slippery surface show that all legs can make active retraction movements after a connective is severed, but for caudal ipsilateral legs the vigor of both protraction and retraction is reduced. In the current experiments, the retraction movement imposed by the mechanical coupling of the leg when it contacts the wheel probably facilitates regular stepping.

In the stick insect it appears that this descending excitation for walking is primarily passed through the ipsilateral connectives while less excitation crosses at each ganglion. Thus, the leg immediately caudal to the operation site makes slower and less-steady swing movements. Possibly the target information referred to above also serves to increase the speed of the swing movement and trigger the transition to stance. In the absence of this information the leg often made a relatively rapid protraction at first, but then hung in the air for a while before finally contacting the wheel and beginning a stance. Thus, when the leg caudal to the severed connective paused, it stopped near the end of its swing but, in the free-walking animal, mechanical factors held the leg at the end of its stance.

Some descending excitation crosses in each thoracic ganglion and this excitation, or secondary activity arising from the stepping it induces, is transmitted further to the rear. Hence, following section of the ipsilateral connective in the mesothorax, the rear leg is more active than the middle leg: the rear leg benefits from activity crossing in the mesothoracic ganglion or from the stepping of the middle leg. Unilateral section of both thoracic connectives removes the ipsilateral

links among the three thoracic ganglia, leaving them dependent on contralateral influences for any coordination. The front legs always show normal stepping, as they are not cut off from the descending activation from higher centers. The rear legs are generally more active than the middle legs, indicating either that more activity crosses in the metathoracic than in the mesothoracic ganglion or that the rear legs benefit from activity ascending from the abdomen.

In summary, it appears that the major intersegmental coordinating influences are transmitted from one hemiganglion to the next through the ipsilateral connective. All six mechanisms known to act between ipsilateral legs in the stick insect are affected by cutting the ipsilateral connective. In addition, the general excitation is reduced. The resulting changes are qualitatively greater than those reported for cockroaches. Unlike the adult stick insect, the cockroach is a fast walker, generally employing tripod coordination, so peripheral feedback may be relatively less important than intrinsic neural mechanisms in the generation of the step rhythm. A tighter coupling of the central pattern generators in all directions would provide greater redundancy and better compensation for the loss of one connective. In the stick insect, specific step-by-step control mechanisms appear to play a more important role than in the cockroach; this may be necessary to guarantee accurate stepping in the more complex environment of the stick insect.

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