

STICK INSECT LOCOMOTION ON A WALKING WHEEL: INTERLEG COORDINATION OF LEG POSITION

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SUMMARY

Continuous measurements of anterior-posterior leg position recorded from stick insects walking on a wheel were tested for relationships among spatial and temporal parameters of leg coordination. This analysis revealed that the protraction of middle and rear legs is guided by the ipsilateral front and middle legs respectively. Protraction endpoint for each rear leg shows a significant positive correlation with the simultaneous position of the ipsilateral middle leg (Figs 1, 2; Table 1). An analogous, but somewhat weaker, correlation exists between the protraction endpoint of each middle leg and the position of the ipsilateral front leg.

This coordination of spatial parameters was tested experimentally by manipulating the position of the forward leg. When a middle leg is restrained in various positions, the ipsilateral rear leg adjusts its protraction endpoint accordingly (Fig. 3). However, its retraction endpoint does not undergo parallel shifts; consequently, step amplitude, protraction duration, and step frequency all change as a function of middle leg position. When a sinusoidal movement is imposed on either a middle or front leg, the adjacent, caudal leg continuously adjusts its protraction endpoint according to the momentary position of the forward leg (Fig. 4). This adjustment is again accompanied by changes in step amplitude and step period, changes which may affect all five unrestrained legs.

The anterior-posterior leg position measured in our experiments primarily reflects the angle of the coxo-thoracic joint; this angle is monitored by hair rows and hairplates located on the coxa (Wendler, 1964; Baessler, 1965). Modifying these external proprioceptive inputs revealed both inter- and intrasegmental control functions. The caudally situated hair rows are important for measuring the small variations in the position of the target leg which occur during normal walking. Immobilization of these hairs on a middle leg causes the mean protraction endpoint of the ipsilateral rear leg to shift forward (Fig. 5: o1 versus C) and reduces or eliminates the step by step correlation of this protraction endpoint with middle leg position (Table 1). The additional immobilization of the cranially situated hairplates usually leads to a caudal shift in the protraction endpoint of the ipsilateral rear leg (Fig. 5: o2 versus o1) and reduces any residual correlation (Table 1). The actual position of the protraction endpoint reflects an integration of intersegmental signals representing the

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position of the target leg and intrasegmental signals from the sensory hairs on the protracting leg. Both operations may affect the duration of protraction in both the operated target leg and the adjacent, caudal leg.

INTRODUCTION

Interleg coordination is an intrinsic feature of insect locomotion. Numerous studies of different insect species have described temporal features of this coordination in the form of preferred phase relations and timing restrictions (for reviews, see Wilson, 1966; Bowerman, 1977). These temporal features have been used to construct model networks which postulate specific kinds of interleg interaction (Wendler, 1968; Baessler & Graham, 1978; Graham, 1977; Cruse, 1981). Nevertheless, the intersegmental interactions which can be demonstrated in dissected preparations are quite weak in comparison to intraleg reflexes (Wilson, 1965; Delcomyn, 1971; Graham & Wendler, 1981).

The apparent weakness of interleg reflexes in dissected preparations, plus the realization that intraleg reflexes vary considerably between quiescent and active insects (Baessler, 1976; J. Dean, personal observation), led to our undertaking a further, detailed analysis of the stick insect. This analysis was designed to take advantage of an automated method for registering leg position (Wendler, 1978). Applying regression techniques, the analysis began by seeking relationships among different walking parameters which might explain some of the variability apparent in individual parameters. The first part of this report describes one such relation which serves to influence the position of leg placement at the end of protraction. The second part of the report shows how this behaviour is affected by externally imposed manipulations. The results confirm and extend findings by Cruse (1979), who used a different approach which confined his investigation to single, discontinuous steps. The third part of the report examines some of the proprioceptive factors involved in this interleg coordination.

METHODS

Data were collected from adult female stick insects (*Carausius morosus*) walking along a light walking wheel (radius 16.4 cm, rim width 3.0 cm, inertial mass equivalent to 1.5 gm). For each experiment, an insect was glued along its dorsal surface to a metal rod which was then clamped at a fixed distance above the wheel. Experiments were conducted in dim light. If the animals did not walk spontaneously, they were stimulated to do so either by puffs of air or light touches to their abdomens.

The anterior-posterior position of each leg was sampled at 10 ms intervals using a photoelectric method described in detail elsewhere (Wendler, 1978). Basically, this method employs a photocell to record the times at which each femur interrupts a light beam which moves alternately on each side of the animal along a path parallel to the body axis. The resulting measurement corresponds to the tangent of the angle between femur and body axis as projected onto a plane perpendicular to the light beam. This measurement principally records movement of the coxo-thoracic joint; it

Proximally proportional to the anterior-posterior tarsus position as long as the femur does not rotate and the lateral position of the tarsus is constant, as it is when the tarsus is always placed on the edge of the wheel surface. However, it has been shown for free walking animals that the relation between tarsus position and femur angle is not linear for extreme leg positions (Baessler, 1972). In our measurement units, the proportionality factor also changes with the lateral position of the light beam: this position varied from one animal to another but remained constant for each animal. A second photoelectric sensor attached to the wheel axis measured the absolute rotation of the wheel in 1 mm units.

These data on wheel motion and leg position were recorded on tape (15 ips FM) in a pulse-coded format and processed later by a PDP 11/40 minicomputer. Using the computer, protraction and retraction endpoints were defined semi-automatically for each leg. (Under the experimental conditions described here, these two endpoints correspond closely with the Anterior and Posterior Extreme Positions – AEP and PEP – used by other authors, e.g. Baessler, 1972, Cruse, 1976, but they were not defined as the extreme leg positions.) Subsequently, regression and phase relations were computed by combining as needed: (1) the position and timing of the protraction and retraction endpoints of interest, (2) the corresponding position of other legs, and (3) the phase of any imposed periodic stimulus. Steps were excluded from this analysis if the period of the right rear leg differed by more than 20% from its value in either the preceding or the subsequent step. The multiple regression programme incorporated IBM SSP subroutines. These subroutines calculated regression coefficients b and their standard errors as well as a Student's t for the null hypothesis: $b = 0$. In addition, they provided the simple correlation coefficient r for one independent variable or the multiple correlation coefficient R for more than one independent variable. R^2 , or r^2 for simple regression, is equal to the fraction of the total variance in the dependent variable which can be attributed to the regression.

Leg restraint was achieved by placing the distal femur through a small wire ring (diameter 1.5 mm) clamped in a manipulator. This arrangement allowed tarsus and tibia to move freely but the femur was held high enough to prevent the tarsus from contacting the wheel. The tarsus was not provided with a substrate; this omission was necessary in order to avoid the disruption caused by the tendency of adjacent legs to grasp such a support instead of the wheel, a tendency which itself reflects the phenomenon to be investigated.

Motion was imposed on a leg by loosely attaching the distal femur to a balsawood arm connected to a pen-motor (Brush Co.). The femur was held high enough to prevent tarsus contact with the wheel while the pen-motor rotated the leg forward and backward around the coxo-thoracic joint. The vertical axis of rotation for the balsawood arm was positioned directly over this joint. The axis of normal joint rotation is slightly inclined from vertical (Graham & Wendler, 1981), but it was necessary to use a vertical axis in order to prevent the apparatus from interfering with the measurement of leg position. This arrangement sufficed to move the tarsus in a horizontal arc just above the substrate and within its normal path during stepping.

Modification of sensory input centred on two sets of mechanoreceptive hairs: (1) four hair rows located on the caudal surface of the coxa (Baessler, 1965) and (2) plates $cxHPv$ and $cxHPd$ located on the cranial margin of the coxa (Wendler, 1964:

here, the labels trHP, cxHPv, and cxHPd are introduced to replace the original designations BF1, BF2, and BF3 of Wendler, 1964; this change is made for the sake of clarity and increased uniformity of terminology, e.g. Pringle, 1938; Pflueger, Braeunig & Hustert, 1981). Depending on joint position, individual hairs in each set are successively covered and deflected by folds of joint membrane. Retraction of the coxa increases the number of deflected hairs in the hair rows; protraction does the same for the hairplates. Phasic input from these hairs was eliminated by first exposing the full set of hairs and then applying a thin layer of wax/rosin mixture to fix the hairs in an upright position. This method necessarily introduced a potential mechanical impediment to the normal movement of the joint.

RESULTS

The results presented here focus primarily on factors influencing the endpoint of leg protraction. A secondary theme concerns consequences of this spatial coordination for other step parameters. Three types of results will be presented in turn: (1) the results of a regression analysis applied to data from intact, unrestrained animals – an analysis which examined naturally occurring variation in protraction endpoint; (2) changes in protraction endpoint caused by restraining one leg or by subjecting it to sinusoidal movement; and (3) results of modifying sensory inputs from leg proprioceptors thought to mediate these effects.

Regression relationships

The regression analysis began with a number of spatial and temporal parameters in a search for regular features of stick insect walking. The clearest result of this search concerned step by step variation in protraction endpoints. The initial finding was a positive correlation between protraction endpoint and its starting position (= endpoint of the preceding retraction).

For the front legs, this was the most common relation but it was not very consistent. Correlation coefficients reached 0.77 but were under 0.40 in most cases and were not significant for about a third of the walks examined.

For rear and middle legs, further tests with additional factors showed that variation in their protraction endpoints was more strongly and consistently correlated with another parameter – the momentary position of the adjacent anterior leg at the time protraction ended. For example, the position of the middle leg at the time that the rear leg completed its protraction typically accounted for between 16% and 75% of the total variance in the rear leg protraction endpoint (r values between 0.4 and 0.85; e.g. Fig. 1 and Table 1: Intact values). In our measurement units for the animals in Table 1 (Intact values) and six others not tabulated, the slope of the regression of rear leg protraction endpoint on simultaneous middle leg position was generally between 0.65 and 0.8; these values remained much the same when the measurements were converted to degrees of arc between femur and body axis. The relation between middle and front legs was weaker than that between rear and middle legs. Variation in the position of the front legs accounted for only 4% to 49% of the total variance in the protraction endpoint of the ipsilateral middle leg (r values between 0.2 and 0.7; e.g. Table

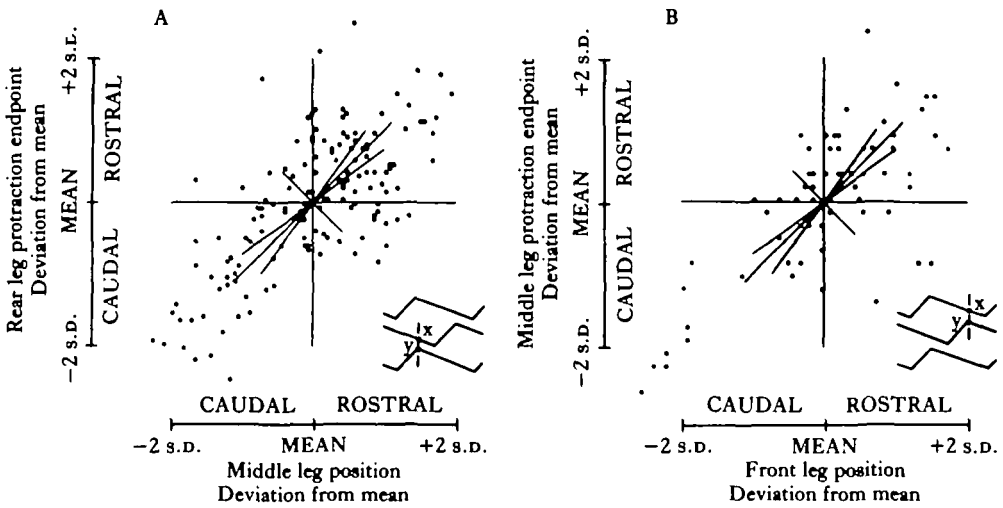


Fig. 1. Regression of leg protraction endpoint on the simultaneous position of the rostral adjacent leg. Each point is taken from a step in a walk by an intact stick insect. The values have been converted into normal variables and plotted as deviation from their means. This automatically places the major axis of the distribution along the 45 degree diagonal. Perpendicular to this major axis is the minor axis. The difference in length of major and minor axes and the acuteness of the angle formed by the two regression lines lying on either side of the major axis indicate the extent of the relation between the two variables. (A) Rear leg protraction endpoint (PRO3) in relation to the simultaneous position of the ipsilateral middle leg (POS2). $N = 148$, $r = 0.74$, $t = 13.14$. (B) Middle leg protraction endpoint (PRO2) in relation to simultaneous position of the ipsilateral front leg (POS1). $N = 69$, $r = 0.73$, $t = 8.7$.

Intact values plus six additional animals not tabulated); regression coefficients were between 0.2 and 0.4 in most walks.*

Despite these appreciable correlations, the link between protraction endpoint and the simultaneous position of the forward leg is not immediately obvious during normal walking because both these parameters vary within quite narrow ranges. The ratio of the standard deviation in step endpoint to mean step amplitude is usually between 8% and 15%. The relation does become obvious when for some reason the target leg is in an unusual position, for example when omitting a protraction (Fig. 2).

Fixed leg experiments: step changes elicited by fixing one leg

Correlation alone does not establish a causal link between variables. However, Fig. 2 aptly illustrates the temporal sequence in which the relevant leg positions are established and thereby suggests the direction of the underlying coordinating influence.

* Additional parameters tested in stepwise multiple regression equations included: step velocity and/or period, protraction starting point (= retraction endpoint) of the anterior adjacent leg, position of the anterior adjacent leg at the time protraction begins, and position of the contralateral leg. For most animals, these additional parameters did not contribute significantly to explaining variance in protraction endpoint, but in some animals one or more either added to or assumed part of the variance attributed in the simple model to the momentary position of the forward adjacent leg. The coefficient for step speed was usually positive (six of seven animals for each leg), but rarely significant (e.g. Table 1), confirming a previous result noted by Wendler (1964). With these additional parameters, the proportion of explained variance sometimes was as high as 81% ($R = 0.90$).

Table 1. *Regression values for normal walking and for walking with modified sensory input*

Operations to modify sensory inputs were performed either (A) on a middle leg or (B) on a rear leg. Regressions were calculated for three conditions of the test leg: (1) the control walk of the unoperated animal (Intact), (2) walking after wax was applied to cover the coxal hair rows (o1), and (3) walking after additional wax was applied to cover hairplates cxHPv and cxHPd (o2). The values given are from multiple regression analyses in which the dependent variable was the protraction endpoint (PRO3 or PRO2) for one ipsilateral leg and the two independent variables were instantaneous position of the adjacent anterior leg (POS2 or POS1) and step speed (vel). For each animal in each condition, the table shows: (1) the partial regression coefficient *b* for the effect of the target leg position on protraction endpoint, (2) the Student's *t* value for the null hypothesis $b = 0$, and (3) the multiple correlation coefficient *R* for the two variable equation. This *R* also represents an upper bound for the simple correlation coefficient *r* between target leg position and protraction endpoint: *r* is approximately equal to *R* except for the four cases (marked with an asterisk) in which the coefficient for speed is significant. Each regression was based on between 25 and 140 steps; *t* values exceeding 2.04 are therefore significant at $P < 0.05$.

Animal	Condition	PRO3 = $b \cdot \text{POS2} + c \cdot \text{vel} + d$			PRO2 = $b \cdot \text{POS1} + c \cdot \text{vel} + d$		
		<i>b</i>	<i>t</i>	<i>R</i>	<i>b</i>	<i>t</i>	<i>R</i>
(A) Operation performed on L2							
A2	Intact	1.28	11.79	0.90	0.23	6.91	0.72
	o1	0.26	2.09	0.30	0.15	0.08	0.32
A3	Intact	0.76	9.03	0.78	0.25	5.60	0.58
	o1	0.72	7.84	0.73	0.22	6.89	0.59
	o2	0.38	3.00	0.65*	0.48	7.03	0.63
A4	Intact	0.80	6.03	0.67	0.30	4.39	0.56
	o1	0.11	0.53	0.35	0.21	1.27	0.23
	o2	-0.20	2.10	0.38	0.07	1.23	0.47*
A7	Intact	0.74	7.07	0.69	0.21	4.47	0.50
	o1	0.37	1.27	0.23	0.08	1.17	0.50*
A8	Intact	0.66	4.06	0.50	0.55	3.73	0.45
	o1	0.82	5.86	0.58	0.59	8.75	0.74
	o2	0.05	0.76	0.23	0.41	2.72	0.28
A9	Intact	0.73	11.68	0.72	0.10	2.59	0.23
	o1	0.27	0.08	0.52*	0.19	4.77	0.55
	o2	0.19	3.50	0.37	0.25	4.77	0.42
A1	Intact	0.70	8.94	0.78	0.37	5.85	0.61
(B) Operation performed on L3							
A5	Intact	0.50	5.70	0.61			
	o1	0.21	1.69	0.23			
	o2	0.08	0.25	0.16			
A6	Intact	1.48	10.40	0.82			
	o1	1.51	9.50	0.77			
	o2	1.59	3.44	0.43			

As the caudal leg protracts towards the adjacent forward leg, the latter is nearing the end of its support phase in a stance which typically was assumed more than half a step cycle previously. Thereafter, the position of this target leg is coupled to that of all the other supporting legs and cannot be varied independently. This temporal order implies that the extent of the protraction is controlled by signals related to the position of the more forward leg.

This hypothesis was tested by imposing different positions on one middle leg a

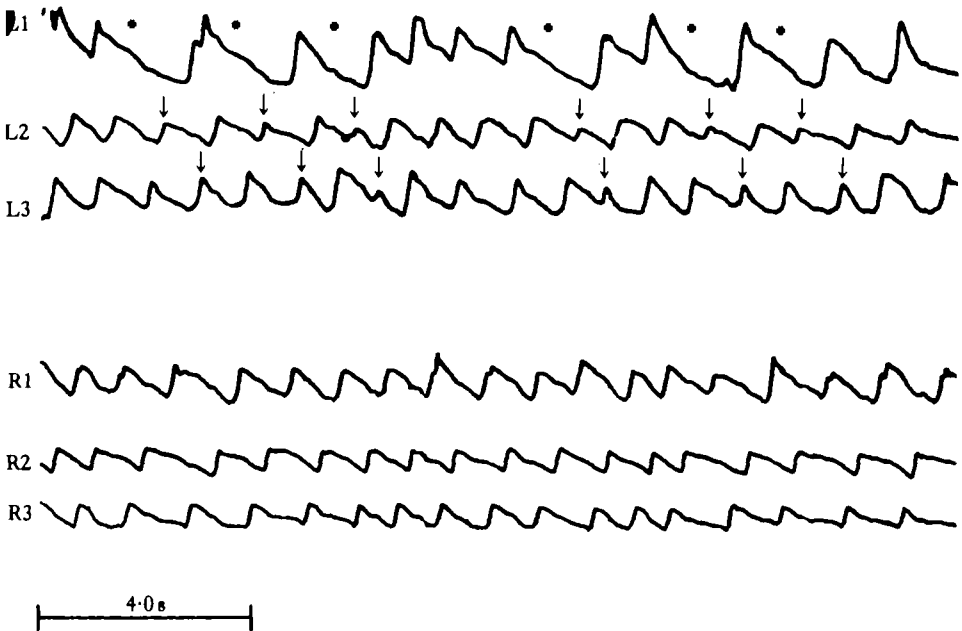


Fig. 2. Omission of front leg protractions leading to adjustment of middle and rear leg protraction endpoints. The figure presents a step sequence in the format recorded by the walking wheel. The six traces represent the positions of the left (L) and right (R) front, middle, and rear legs (1, 2, 3 respectively). Anterior movement of the leg corresponds to upward change in the trace. For each side, the walk can be divided into metachronal cycles of successive rear, middle, and front leg protractions. When the left front leg fails to make a protraction in a cycle (*), the prolonged retraction places it in an extreme posterior position for the next protraction of the ipsilateral middle leg. To compensate, the middle leg shortens its protraction (arrows) which causes it to be in a more posterior position for the next rear leg protraction. The rear leg in turn shortens its protraction (arrows).

observing the stepping of the unrestrained legs, especially that of the ipsilateral rear leg. In the unrestrained animal, the normal position of the middle leg when the rear leg completes protraction is slightly in front of its retraction endpoint. Restraining the middle leg behind the mean retraction endpoint caused the ipsilateral rear leg to shorten its protraction (Fig. 3: PRO3 for fB = fixed back versus C = control). Observation of the animals during the experiment revealed that the rear leg was not blocked by the middle leg. In most steps, the rear leg completed its protraction and stepped down without hesitation and without touching the middle leg. Only occasionally did the rear leg rotate and stroke down across the distal tibia of the restrained middle leg, sometimes repeating the stroke before stepping down behind the middle leg and beginning retraction.

Moving the middle leg forward caused the rear leg to protract farther forward, but extreme forward positions elicited smaller shifts in rear leg protraction than did intermediate positions (Fig. 3: PRO3 for middle leg conditions fM and fF, fixed middle and forward, versus fB, fixed back).

These changes in protraction endpoint occurred without parallel changes in retraction endpoint. On the contrary, for three of the six animals the rear leg retraction continued farther to the rear when the middle leg was fixed farther forward. In all

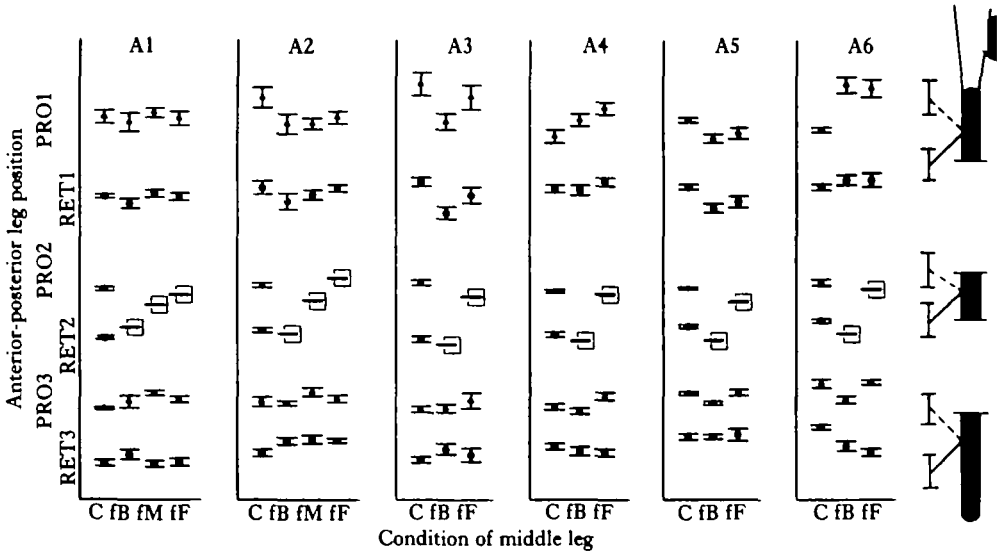


Fig. 3. Protraction and retraction endpoints for ipsilateral legs when the middle leg is restrained in anterior and posterior positions. The ordinate corresponds to that of Fig. 2 for one side of the animal; it represents anterior-posterior femur position in relation to the insect's body axis. Anterior is upward. For the control walk (C), mean protraction (PRO) and retraction (RET) endpoints are given for each leg. The bars indicate three times the standard error for each mean. When the middle leg is fixed for the entire walk in the back, middle, or front part of its range (fb, fM, fF respectively), its fixed position is shown by the single bar. Each part of the figure is derived from a single animal; the letter A plus a number at the top of each part identifies each animal as it appears in Table 1.

Table 2. Protraction amplitude and duration for the ipsilateral rear leg as a function of the position of middle leg restraint

Protraction amplitude is given in standardized units as illustrated in Fig. 3; duration is given in ms. Values are presented for restraint of the middle leg at the back (fb), middle (fM), and front (fF) of its normal step range. All amplitude differences are significant at $P < 0.02$; all duration differences except those indicated by brackets are significant at $P < 0.05$. For five of the six animals, the duration of protraction was significantly correlated with its amplitude during the control walk; for all six animals, the correlation was significant when both control walking and walking with the middle leg restrained in different positions were included.

Animal	L2 Position	L3 Amplitude		L3 Duration		N	
		Mean	s.e.	Mean	s.e.		
A1	fb	26.8	1.0	169.8	11.3	33	
	fM	34.8	0.7	222.3		5.1	98
	fF	30.7	0.9	173.9		7.2	87
A2	fb	18.6	0.7	102.8	5.0	47	
	fM	23.9	0.7	142.9	7.5	42	
	fF	21.3	0.8	151.0	5.4	74	
A3	fb	19.8	0.7	126.3	7.8	42	
	fF	26.7	1.2	180.2	7.6	38	
A4	fb	19.5	0.9	160.9	6.3	60	
	fF	27.4	0.7	160.2	5.2	88	
A5	fb	16.9	0.5	131.8	5.7	99	
	fF	21.1	1.0	159.6	9.0	24	
A6	fb	21.6	0.9	160.1	6.3	119	
	fF	30.8	1.0	145.0	6.8	52	

Animals, the net result was an increase in mean step amplitude for the ipsilateral rear leg (Table 2). This amplitude change for rostral versus caudal middle leg restraint amounted to 20–40% of the control step amplitude. This increase in amplitude usually was accompanied by an increase in mean protraction duration (Table 2).

Restraint of a middle leg also affected the stepping of the ipsilateral front leg. Restraint in the caudal part of the middle leg's range elicited changes which differed from one animal to another. In four of the six animals, both the retraction and protraction endpoints shifted caudally by similar amounts, leaving step amplitude unchanged (Fig. 3). The other two animals showed a rostral shift of protraction endpoint which exceeded the shift in retraction endpoint, leading to an increase in step amplitude. Although protraction amplitude did not decrease for any animal, its mean duration decreased for all six.

Changing the position of the restrained middle leg elicited parallel changes in both protraction and retraction endpoints of the ipsilateral front leg for five of the six animals. Step amplitude was unaltered in all, but protraction duration remained shorter than that of control walking.

Such a differential change in the step amplitudes of ipsilateral rear and front legs must also alter step timing. When one leg decreases its step amplitude relative to that of another, it must either spend more time in protraction or it must step more often. Table 2 shows that for most animals protraction duration decreased for the smaller amplitude steps taken during caudal middle leg restraint; therefore, it can be expected that the rear leg must step more frequently. This expectation was realized: the ipsilateral rear leg stepped at a higher frequency relative to the ipsilateral front leg when the middle leg was restrained in caudal positions and this change was reversed when the middle leg was moved rostrally (Table 3).

Restraining one middle leg in different positions did not systematically affect the step characteristics of the contralateral legs. As a result, changes in the step amplitude and step frequency of the ipsilateral rear leg were reflected in its step frequency relative to the contralateral rear leg (Table 3: L3/R3). Normally the two rear legs make roughly the same number of steps. However, restraining one middle leg in a caudal position increased the relative step frequency of the ipsilateral rear leg (Table 3: L3/R3). This change was reduced or reversed when the restrained middle leg was placed in a rostral position. Since the step amplitude of the ipsilateral front leg did not change with the position of the restrained middle leg, there was at first no reason to expect changes in its step frequency relative to the contralateral front leg. Nevertheless, this pair did show alterations opposite to those of the two rear legs (Table 3: L1/R1). Evidently, the alteration in rear leg stepping, plus possible direct influences of the restrained middle leg, created distortions which altered the step ratios of several other leg pairs. On the contralateral side, the middle and rear legs continued to step virtually one to one. Relative to these two legs, the contralateral front leg in three animals slightly increased its step ratio for caudal middle leg restraint. However, most of the change in step ratio for the front legs apparently reflected an alteration relative to the contralateral side as a unit.

The previous experiments revealed discrete changes in leg coordination when one leg was fixed at different positions for each walk. A further set of experiments demonstrated the continuous nature of these changes by altering the position of the

restrained leg within a walk. For these tests, a single middle or front leg was moved sinusoidally through its step range. Two frequencies of imposed movement were tested: one was about one third that of normal stepping, the second was considerably slower. As expected from the previous results, movement of one middle leg at both frequencies elicited parallel shifts in the protraction endpoint of the ipsilateral rear leg (Fig. 4A, B). However, the retraction endpoint of the rear leg showed smaller, less consistent, shifts. In some step sequences, the rear leg shortened its retraction and made quicker, smaller steps when the middle leg was to the rear. At other times, its retraction endpoint moved in parallel with the protraction endpoint and step period remained nearly constant. This continual variation in step amplitude caused changes in timing indicated by the failure of the rear leg to step one to one with the other unrestrained legs.

Imposing a slow sinusoidal movement on one front leg elicited a similar pattern of changes in the ipsilateral middle leg (Fig. 4C). As expected the protraction endpoint of this leg followed the momentary position of the front leg. The retraction endpoint showed parallel shifts of lesser magnitude, leading to an expansion of step amplitude for rostral positions of the front leg. Unlike the result for movement of the middle leg, these oscillations in middle leg step amplitude and period were replicated in the remaining legs and all five unrestrained legs maintained one to one stepping.

Table 3. *Ratio of number of protractions for selected leg pairs according to the position in which the left middle leg was restrained*

Step sequences or steps were selected first according to the criterion that the period of the contralateral rear leg was regular. Then the numbers of steps taken in these sequences by the remaining legs were tallied and expressed as ratios. Only protractions crossing the middle position of the leg were counted. The restrained middle leg is L2. L1/L3 is the ratio of ipsilateral front leg to rear leg steps. R3/L3 is the ratio for the two rear legs and R1/L1 is that for the two front legs. The + and - entries express the relative change for walks with L2 restrained caudally versus L2 free (column d1) and for L2 restrained in a rostral versus a caudal position (column d2).

Animal	L2 Condition	L1/L3	d1	d2	R3/L3	d1	d2	R1/L1	d1	d2	N
A1	Free	1.02			0.92			0.98			54
	fB	0.97	-		0.97	+		1.49	+		33
	fM	1.33		+	1.33		+	1.02		-	134
	fF	1.26			1.20			1.13			116
A2	Free	0.85			0.96			1.25			40
	fB	0.69	-		0.71	-		1.44	+		31
	fM	0.93		+	1.05		+	1.17		-	41
	fF	1.01			0.99			1.06			75
A3	Free	1.11			1.00			0.98			62
	fB	0.66	-		0.86	-		1.38	+		29
	fF	0.92		+	1.05		+	1.12		-	36
A4	Free	1.08			1.00			0.96			52
	fB	0.83	-		0.84	-		1.12	+		53
	fF	0.94		+	1.00		+	1.06		-	95
A5	Free	0.98			0.98			0.98			57
	fB	0.79	-		0.87	-		1.27	+		79
	fF	1.00		+	1.00		+	1.15		-	24
A6	Free	0.95			0.92			1.19			57
	fB	0.77	-		0.80	-		1.13	-		101
	fF	0.90		+	0.94		+	1.10		-	65

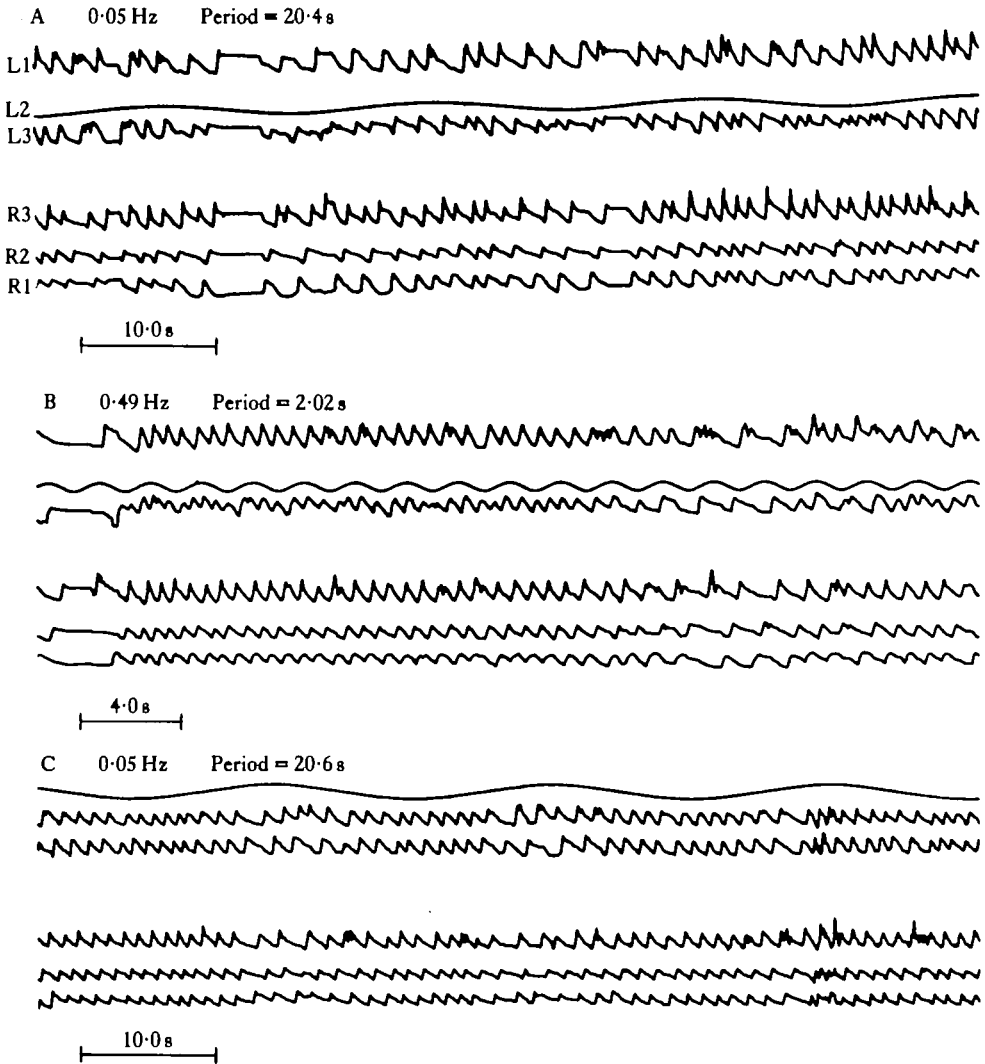


Fig. 4. Changes in protraction endpoint for sinusoidal movement imposed on one leg. As in Fig. 2, the six traces represent the movement of the six legs. The restrained leg is identifiable by its regular, sinusoidal movement. Walking is at times uneven, but for both slow and fast movement of a leg the continual adjustment of protraction endpoint by the adjacent caudal leg is evident. During the caudal portion of the restrained leg's cycle, the caudal ipsilateral leg tends to make quicker, smaller steps. For movement imposed on one front leg, these linked changes in step amplitude and period are transferred to all five unrestrained legs (C).

Role of proprioceptors

The previous results have established that a leg adjusts its protraction endpoint according to the location of the adjacent forward leg. To accomplish this adjustment, the central nervous system (CNS) needs information on the position of the target leg and possibly that of the protracting leg as well. Information on the coxal angle is available from two groups of external proprioceptors located on the coxa itself: four rows of mechanoreceptive hairs spread along its caudal surface (Baessler, 1965) and

two hairplates found near the cranial, proximal margin (Wendler, 1964). (Internal chordotonal organs may provide additional information on joint angle, Hustert, 1978; Braeunig, Hustert & Pflueger, 1981.) These external proprioceptors are known to function in intraleg control loops. Ablation of the hairplates results in over-extension of protraction (Wendler, 1964); ablation of the hair rows, in conjunction with bending the hairs of the hairplates, disturbs the proper completion of retraction (Baessler, 1977). Although Baessler noted frequent overstepping by the rear leg adjacent to an operated middle leg, neither of these previous studies explicitly measured changes in adjacent, unoperated legs.

The last set of experiments examined interleg coordination of stepping when input from the external proprioceptors was altered. For each animal, proprioceptive input was modified in two stages. In the first operation (o1), wax was applied to the four coxal hair rows; in the second (o2), additional wax was added to cover the hairplates cxHPv and cxHPd. Three possible functions of the sensory input were tested.

The first function to be considered was whether sensory information from the target leg is involved in setting the average protraction endpoint of the adjacent caudal leg. The hypothesis was as follows. Fixing the caudal hair rows in an upright position (o1) should remove an input which normally signals increasing retraction. Therefore, the CNS should attribute to that leg a position forward of its actual position. Based on this faulty assessment, an operated middle leg should retract farther to the rear and the ipsilateral rear leg should protract farther forward. Only the second part of this prediction was consistently fulfilled. In all six animals, the rear leg's protraction endpoint shifted forward (Fig. 5: PRO3 for o1 versus C), despite the fact that the corresponding position of the middle leg generally remained unchanged (Fig. 5: upper axes). The rear leg's retraction endpoint moved forward in all six animals. Step amplitude did not show consistent changes but protraction duration was significantly increased in four animals (Fig. 6: L3 duration for o1 versus C). The predicted caudal shift in the middle leg's retraction endpoint only occurred for three animals. However, the middle leg's protraction endpoint moved caudally in five of the six animals. The net effect was a shortened step amplitude in all six animals (Fig. 6: L2 amplitude for o1 versus C). Nevertheless, in three of the six, the operated leg required a longer time for this smaller protraction (Fig. 6: L2 duration for o1 versus C). Removal of hair row input alone sometimes interfered with the proper termination of retraction. In these steps, the operated middle leg failed to release the wheel upon reaching a posterior position. Instead it was pulled free by a build-up of force from the other legs (Fig. 7).

Subsequent immobilization of the rostral hairplates (o2) introduced contradictory signals in that absence of activity from the hairplates normally indicates caudal leg positions whereas the continuing absence of activity from the caudal hair rows indicates forward positions. It was expected that this second operation would counteract any bias induced by the first operation and cause middle and rear legs to revert towards their normal step relations. For all four animals, the mean protraction endpoint of the operated leg shifted forward relative to values for o1 as expected (Wendler, 1964); the retraction endpoint moved forward for three animals (Fig. 5: PRO2 and RET2 for o2 versus o1). Extreme overstepping, which follows ablation of the hairplates alone (Wendler, 1964), did not occur. Step amplitude was slightly increased in all four animals and protraction duration remained longer than control values (Fig. 6). The

Ipsilateral rear leg reacted as expected in three of the four animals: its protraction Endpoint shifted caudally despite the rostral shift in the corresponding position of the middle leg (Fig. 5: PRO3 for o2 versus o1). In all four animals, rear leg protraction duration was longer than in control walking (Fig. 6) and retraction endpoints remained forward of control values (Fig. 5).

The second feature of interleg coordination likely to depend upon control signals from the target leg was the step by step coordination of protraction endpoint. For the same animals described above this feature was studied using both a regression analysis to test within the small range of variation in normal walking and leg restraint to test a wider range of target positions. In four of the six animals, immobilizing the four caudal hair rows alone was sufficient to reduce the correlation between middle leg position and rear leg protraction endpoint (Table 1: o1 values). In the two exceptions, subsequent immobilization of the hairplates reduced the remaining correlation (Table 1: o2 values). After the fixation of the hair rows, large changes in the leg's position caused by restraint near the forward and rear margins of its step range were still conveyed to the rear leg (three animals tested). After subsequent fixation of the hairplates, even these large differences in middle leg position had little or no effect on the protraction endpoint of the rear leg (two animals tested).

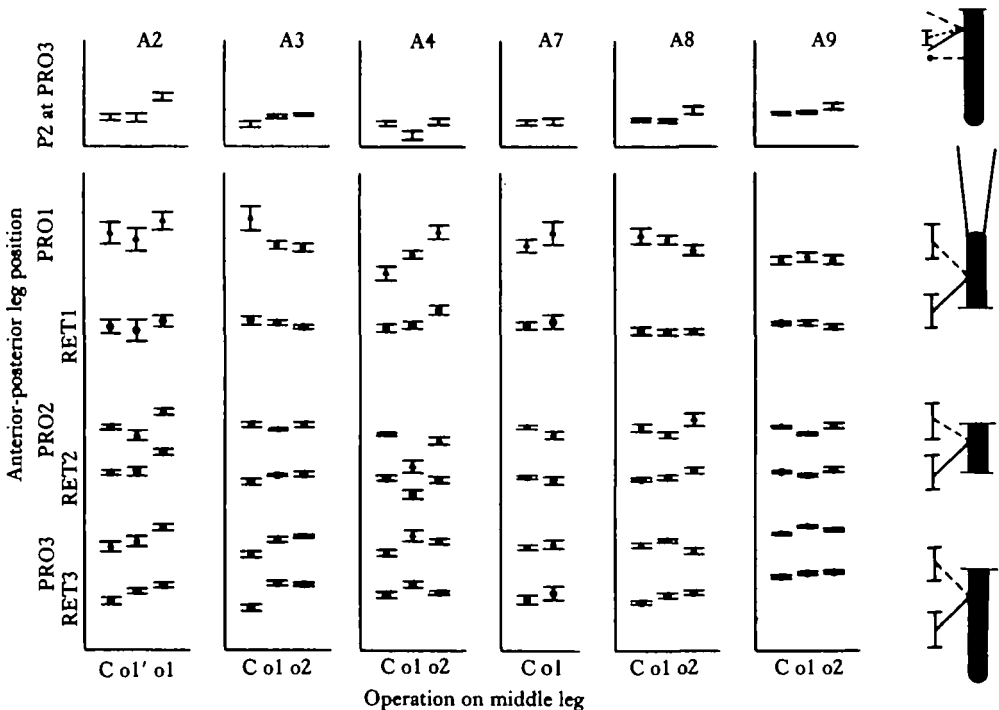


Fig. 5. Protraction and retraction endpoints for ipsilateral legs following removal of sensory inputs from the coxa of the middle leg. The format for the lower part of the figure is identical to that for Fig. 3. The upper part of the figure repeats a portion of the ordinate in order to show the position of the operated middle leg at the time the ipsilateral rear leg completes its protraction. Each part presents the results from one animal; these are identified as they appear in Table 1. For each animal, means and $3 \times$ s.e. are plotted for the control walk (C), walking after wax had been applied to the caudal hair rows (o1), and after additional wax had been applied to the rostral hairplates cxHPv and cxHPd (o2). For animal A2 only the three dorsal hair rows were covered in the first operation (o1').

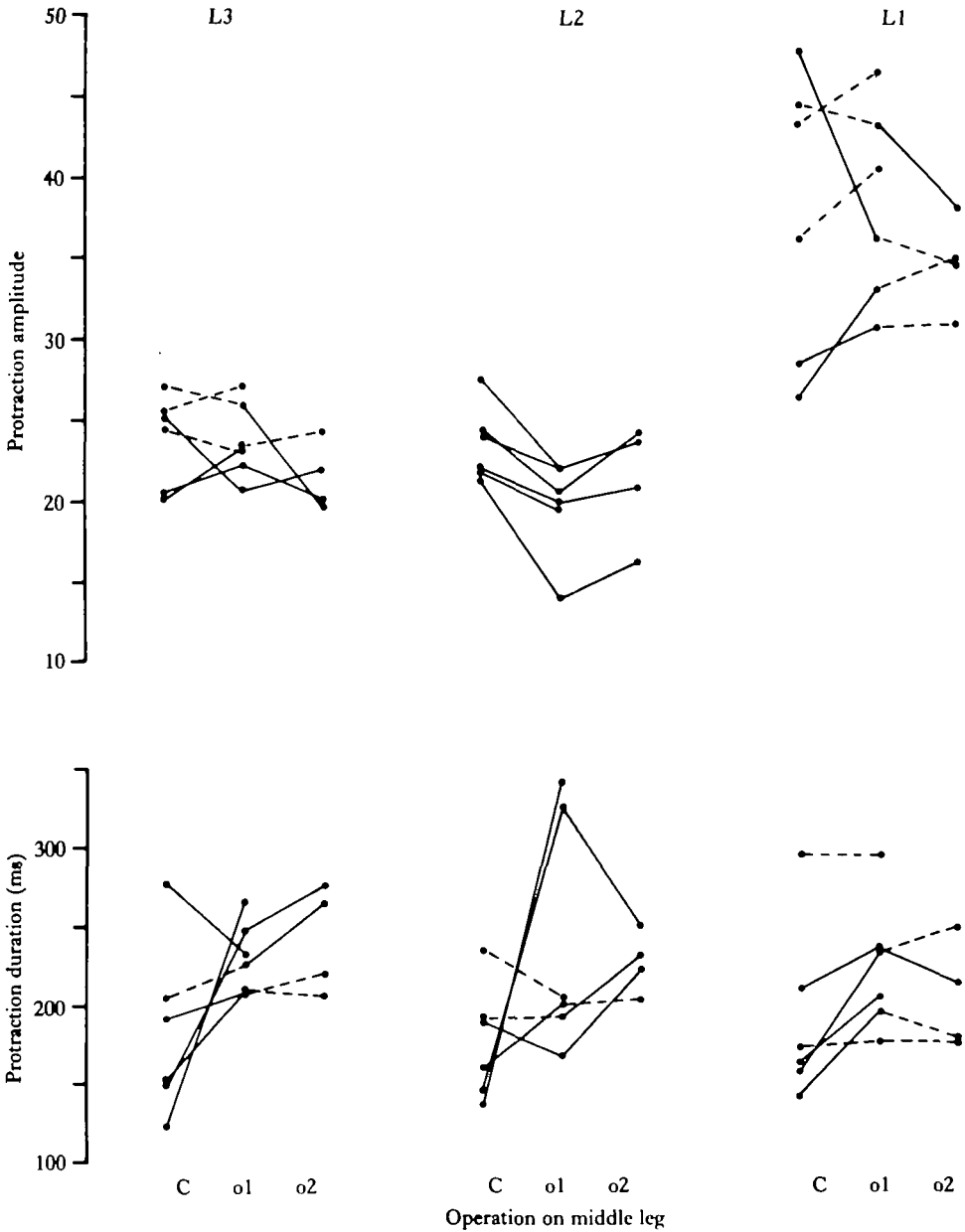


Fig. 6. Protraction amplitude and duration of ipsilateral legs as a function of proprioceptive input from the middle leg. The top row of the figure shows protraction amplitude in normalized units; the bottom row shows protraction duration in ms. Values are plotted for left front, middle, and rear legs (L1, L2, and L3, respectively). The three conditions of the left middle leg are as follows: (1) intact for the control walk (C), (2) wax covering the caudal hair rows (o1), and (3) additional wax covering the rostral hairplates (o2). Measurements for each individual animal are connected by lines. Only the mean values are plotted but the significance of any change induced by the operation is indicated by the connecting lines. Solid lines connect means which differ at $P < 0.05$ (t test); dashed lines connect means which do not differ at this level.

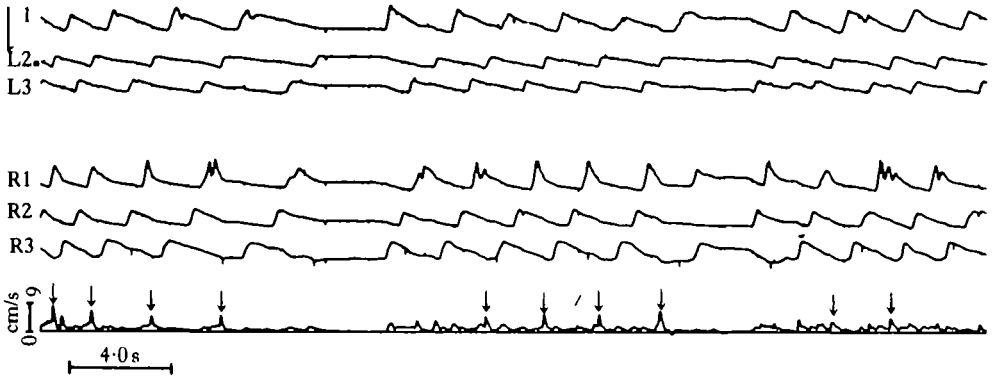


Fig. 7. Sample walk showing an operated middle leg's failure to release at the end of retraction. The format is that of Fig. 2 with the addition of a velocity record derived from the movement of the wheel. The caudal hair rows of the left middle leg were covered with wax, causing the leg occasionally to fail to release the wheel properly at the end of its retraction. The resistance then provided by the middle leg leads to a build-up of force in the other supporting legs which is revealed by the velocity spurt (arrows) when the grasp of the middle leg is broken.

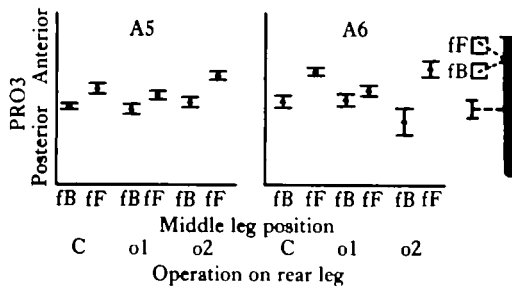


Fig. 8. Ipsilateral rear leg protraction endpoint for front and back positions of a restrained middle leg: effect of removing sensory hair input to the rear leg. The ordinate is a portion of the full ordinate of Fig. 3. Mean and $3 \times$ s.e. are shown for protraction endpoint only. Three pairs of measurements were made: first, on the intact animal before any operation (C); second, after application of wax to the caudal hair rows of the rear leg (o1); and finally, after application of additional wax to its rostral hairplates cxHPv and cxHPd (o2). For each condition, the protraction endpoint was measured for restraint of the ipsilateral middle leg in front (fF) and back (fB) extremes of its range. Removal of hair row inputs from the rear leg shortens its forward protraction and reduces the total change for a given change in middle leg position. Subsequent removal of hairplate input allows greater forward protraction and increases the total change.

The third feature of interleg coordination to be investigated concerned the contribution of the protracting leg's own proprioceptors to its adjustment to different target positions. This function was tested using the animals described above by examining the correlation results for the leg pair in which the operated middle leg served as protracting rather than as target leg. In two additional animals, proprioceptors on a rear leg were modified. Results varied among the eight animals but half continued to show a significant correlation between protraction endpoint of the operated leg and the position of the adjacent forward leg (Table 1: five of eight animals after o1, four of six animals after o2). For the two animals with modified rear leg output, the protraction endpoint of this leg was also examined in relation to restraint

of the ipsilateral middle leg. Neither operation eliminated the adjustment of the rear leg's protraction endpoint in response to restraint of the ipsilateral middle leg in extreme positions. However, signals from the two sets of receptors interacted in opposite ways with signals from the target leg. Absence of hair row input opposed large rostral protractions and decreased the change in endpoint resulting from a fixed change in the position of the middle leg (Fig. 8: o1 fB and fF). Subsequent immobilization of the hairplates removed this bias and permitted larger than normal changes in protraction endpoint for the same change in middle leg position (Fig. 8: o2 fB and fF).

DISCUSSION

The results described here demonstrate that during locomotion the endpoint of middle and rear leg protraction is guided by the instantaneous position of the adjacent forward leg. Our method of recording leg position only measured the angles of the coxo-thoracic joints in the target and protracting legs. However, the function of this guiding response is only clear when its effect on tarsus placement is considered. Observation of animals walking on the wheel as well as inspection of the footprints of free-walking insects shows that each trailing leg steps to a position slightly behind that of the leg in front of it: middle and rear legs in turn step close to the footprints of the ipsilateral front leg (Wendler, 1964). Cruse (1979) demonstrated an active mechanism for this phenomenon. He began with a stick insect standing with one middle leg on an unmoving platform and the other five legs on a treadwheel; he then recorded the position to which the tarsus of the ipsilateral rear leg moved in its initial protraction. By altering the position of the platform in relation to the insect's body, he demonstrated that the rear leg adjusted both the lateral and longitudinal aspects of its protraction in order to place its tarsus a constant distance behind that of the middle leg. Our measurements demonstrate that this same guiding response occurs during continuous locomotion. During walking, a forward leg typically is nearing the end of its support phase when the leg behind it finishes protraction; therefore, the effect of the adjustment in protraction endpoint is to direct each leg to a location where the more rostral leg has already found a supporting substrate.

In our experiments, the protracting leg did not compensate fully for changes in target leg position. With few exceptions the regression coefficients were less than one; they were much less than one for the relation between middle and front legs. These coefficients suggest that compensation is less than complete; however, this interpretation of the results is subject to three qualifications. First, our method recorded only the protraction of the coxo-thoracic joint; this is only one of the movements involved in tarsal placement. Our measurement is strictly proportional to longitudinal tarsus position only as long as there is no rotation of the leg and the tarsus steps to the same lateral position. Neither condition is strictly obeyed. Baessler (1972) found that for free walking stick insects, tarsus position is linearly related to femur angle only in the middle range of step movement. Deviations can be expected for extreme positions, such as the protraction and retraction endpoints measured in our experiments. Second, the curvature of the wheel, the restriction of lateral movement of the body, and the removal of the need for the insect to support its own weight may all redu-

the compensation which might normally be expressed. Third, each leg normally avoids stepping on or in front of the leg ahead of it; this is a reasonable precaution to avoid obstructing the ensuing protraction of the forward leg. If the protracting tarsus does contact the adjacent leg, it immediately withdraws and moves to the rear (Graham, 1979). Therefore, the errors in compensation can be larger in the caudal direction. The degree of compensation as measured by a regression analysis will be reduced because the variance is not constant for all target locations.

Deviation from complete compensation was also evident in the experiments in which a middle leg was restrained in extreme points of its range. Here the method of restraint may well have hindered optimal performance. The lack of restraint on the tibia allowed considerable variation in the tarsus position which probably is the actual target of the protracting leg. Moreover, the target leg distal to the femur did not signal the presence of any substrate. Nevertheless, partial compensation did occur and the experimental conditions should be amenable to neurophysiological investigation of the underlying neural mechanism. The alternative method of providing the target leg with a small platform has the disadvantage that the very guiding response under study brings the posterior leg onto the platform and thereby disrupts regular walking.

Information on the angle of protraction at the coxo-thoracic joint is provided by two sets of coxal sensory hairs. These sense organs participate in both intra- and inter-segmental control pathways. As might be expected from their positions on opposite sides of the coxa, they have antagonistic influences in both pathways. In the intact animal, absence of activity from the caudal hair rows is associated with forward positions of the target leg and forward placement of the protracting leg, while absence of activity in the rostral hair plates is associated with posterior positions. By successively immobilizing the sensory hairs in a layer of wax, we hoped to leave the sense organs intact while biasing the total input to the CNS so as to signal an incorrect leg position. In retrospect, this bias might better have been accomplished by shaving the sensory hairs. With our method, the presence of the wax between the moving joint surfaces may have contributed to the large variation in results from one animal to the next.

The intrasegmental pathway has been investigated previously. Signals from the hairplates actively oppose exaggerated forward protraction (Wendler, 1964) while signals from hair rows and hairplates together act to terminate retraction properly (Baessler, 1977). The present results largely agree with these previous findings. Two differences probably relate to differences in method. First, immobilization of both hairplates and hair rows did not lead to the extreme overstepping found by Wendler (1964) after ablation of the hairplates alone. Second, applying wax to the hair rows did not elicit the consistent caudal extension of retraction which Baessler (1977) obtained by ablating these hairs and simultaneously applying paste to bend the hairs of the hairplates forward. Apparently this false signal from the hairplates must be present and sufficiently large to achieve a consistent result (U. Baessler, personal communication).

The intersegmental signals derived from the coxal proprioceptors perform two related functions. One is to monitor target leg position to enable the step by step control of the protraction endpoint of the adjacent caudal leg. During normal walking, the target position only varies over a small, caudal fraction of the full step range. The

caudal hair rows are important for monitoring this variation, since immobilizing the hairs on the target leg usually decreased the compensation shown by the protracting leg. The cranially located hairplates do signal larger changes in target position which extend into the forward part of the leg's range.

The second intersegmental function is properly speaking another facet of the step by step regulation. It is expressed as a bias in the mean protraction endpoint of the protracting leg. Experimental removal of hair row input from the target leg resulted in a rostral shift in mean protraction endpoint of the adjacent caudal leg but did not usually alter the corresponding position of the target leg. This combination sometimes produced overstepping of the kind observed by Baessler (1977). Additional immobilization of the hairplates caused this protraction endpoint to revert to more caudal positions and at the same time caused the target leg position to shift forward. This latter change may indicate that the hairplate input contributes to a position dependent signal affecting the timing of protraction by the caudal leg. One possible interpretation would be that the absence of hairplate input, which normally signals posterior positions of the target leg, is one factor promoting protraction of the adjacent leg.

Both these intersegmental influences probably are conveyed by interneurons rather than by direct interganglionic projections of the sensory hairs. In cockroaches, the coxal hairplate is known to project into segmental interneurone pools (Pearson, Wong & Fournier, 1976). In locusts, the only intersegmental sensory projections are those of the proximal chordotonal organs (Hustert, 1978; Braeunig *et al.* 1981). The latter authors report unpublished results showing that the projection pattern in the stick insect is similar. Preliminary cobalt labelling in our laboratory has not shown any intersegmental projections from hairplates or hair rows. The chordotonal organs described by Hustert (1978) are capable of registering coxal protraction. These organs were left intact in our experiments but they apparently are incapable of sustaining a guiding response to any significant degree.

In the absence of signals from its own hairplates and hair rows, a leg can still adjust its protraction endpoint to changes in the position of its target leg. Intra- and interleg influences interact to determine protraction endpoint. Wendler (1964) showed that signals from the hairplates act to damp forward protraction and to resist both external and internal disturbances tending to move the leg forward. The present results show that one of the internal signals subjected to this damping is derived from the position of the adjacent forward leg. The influence of this particular signal is limited to one part of the leg's step – the endpoint of protraction. In cockroaches, an analogous function is performed by the coxal hairplate (Wong & Pearson, 1976); this hairplate projects both onto leg motoneurons and onto segmental interneurons (Pearson *et al.* 1976). Integration with intersegmental signals could occur at either level.

This control signal arising from the forward adjacent leg represents a spatial coordinating signal which passes from front to rear. This direction is opposite to that proposed in most models for temporal coordinating signals of insect walking (Wendler, 1968; Graham, 1977; Wilson, 1966), but Cruse (1981) has shown the feasibility of a model based on front to rear flow of temporal cues. His model incorporates both temporal and spatial parameters of leg movement; it includes the spatial coordination demonstrated in the present results, but this feature is not a major determinant of the temporal pattern.

During normal walking, the small changes in protraction amplitude due to variations in the position of the forward leg are normally accomplished without a measurable effect on protraction duration or step rate. When the spatial changes are made more extreme, the consequences for temporal coordination become more evident. This interaction of spatial and temporal parameters is dramatically revealed when one front leg is subjected to a slow, continuous change in position (Fig. 4C). Two further examples appear in the comparison of walking for different positions of middle leg restraint. Changes in the mean protraction amplitude of the ipsilateral rear leg are directly coupled to changes in mean protraction duration and inversely coupled to changes in step rate. An additional role for sensory input in modifying the speed of protraction is suggested by the increase in protraction duration following removal of sensory hair input.

In summary, this report has discussed adjustments in leg placement during locomotion which are guided by the position of the forward adjacent leg. However, this is only one circumstance in which it may be necessary or advantageous for the movement of one leg to be adjusted according to the position of another. Therefore, the CNS should be able to monitor the position of each leg and to integrate this information into the control of the other legs in a manner appropriate to such different tasks as locomotion, cleaning, and searching. One circumstance in which this plasticity might be sought is when a stick insect walks backwards. With the change in direction, a middle leg searching for a substrate at the end of its swing needs to be guided not by the ipsilateral front leg but by the rear leg. Backward walking is unusual in the insect's normal life; it is more common on a walking wheel but does tend to be slow and irregular. In one instance in which a stick insect made enough regular steps to allow a regression analysis, the placement of the middle leg at the end of its swing was significantly correlated with the momentary position of the ipsilateral rear leg.

This plasticity of interleg coordination may explain the weakness of interleg reflexes which have been found in restrained and dissected preparations. The influence of one leg on the legs adjacent to it is not an invariant factor; it must change according to the behavioural requirements. During locomotion it may only be active during a specific part of a leg's step cycle.

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REFERENCES

- BAESSLER, U. (1965). Proprioceptoren am Subcoxal- und Femur-Tibia Gelenk der Stabheuschrecke *Carausius morosus* und ihre Rolle bei der Wahrnehmung der Schwerkrafttrichtung. *Kybernetik* **2**, 168–193.
- BAESSLER, U. (1972). Zur Beeinflussung der Bewegungswiese eines Beines von *Carausius morosus* durch amputation anderer Beine. *Kybernetik* **10**, 110–114.
- BAESSLER, U. (1976). Reversal of a reflex to a single motoneuron in the stick insect *Carausius morosus*. *Biol. Cybernetics* **24**, 47–49.
- BAESSLER, U. (1977). Sensory control of leg movement in the stick insect *Carausius morosus*. *Biol. Cybernetics* **25**, 61–72.
- BAESSLER, U. & GRAHAM, D. (1978). Zur Kontrolle der Beinbewegungen bei einem laufenden Insekt. In *Kybernetik 1977*, (eds. G. Hauske & E. Butenandt), pp. 54–65. Muenchen, Wien: Oldenbourg.

- BOWERMAN, R. F. (1977). The control of arthropod walking. *Comp. Biochem. Physiol.* **56A**, 231-247.
- BRAEUNIG, P., HUSTERT, R. & PFLUEGER, H. J. (1981). Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. I. Morphology, location and innervation of internal proprioceptors of pro- and metathorax and their central projections. *Cell Tissue Res.* **216**, 57-77.
- CRUSE, H. (1976). The function of the legs in the free walking stick insect, *Carausius morosus*. *J. comp. Physiol.* **112**, 235-262.
- CRUSE, H. (1979). The control of the anterior extreme position of the hindleg of a walking insect, *Carausius morosus*. *Physiol. Entomol.* **4**, 121-124.
- CRUSE, H. (1981). A quantitative model of walking incorporating central and peripheral influences. II. The connections between the different legs. *Biol. Cybernetics* **37**, 137-144.
- DELCOMYN, F. (1971). Computer aided analysis of a locomotor leg reflex in the cockroach *Periplaneta americana*. *Z. vergl. Physiol.* **74**, 427-445.
- GRAHAM, D. (1977). Simulation of a model for the coordination of leg movement in free walking insects. *Biol. Cybernetics* **26**, 187-198.
- GRAHAM, D. (1979). Effects of circum-oesophageal lesion on the behaviour of the stick insect *Carausius morosus*. II. Changes in walking co-ordination. *Biol. Cybernetics* **32**, 147-152.
- GRAHAM, D. & WENDLER, G. (1981). The reflex behaviour and innervation of the tergo-coxal retractor muscles of the stick insect *Carausius morosus*. *J. comp. Physiol.* **143**, 81-91.
- HUSTERT, R. (1978). Segmental and interganglionic projections from primary fibres of insect mechanoreceptors. *Cell Tissue Res.* **194**, 337-351.
- PEARSON, K. G., WONG, R. K. S. & FOURTNER, C. R. (1976). Connexions between hair-plate afferents and motoneurons in the cockroach leg. *J. exp. Biol.* **64**, 251-266.
- PFLUEGER, H. J., BRAEUNIG, P. & HUSTERT, R. (1981). Distribution and specific central projections of mechanoreceptors in the thorax and proximal leg joints of locusts. II. The external mechanoreceptors: Hair plates and tactile hairs. *Cell Tissue Res.* **216**, 79-96.
- PRINGLE, J. W. S. (1938). Proprioception in insects. III. The function of the hair sensilla at the joints. *J. exp. Biol.* **15**, 114-131.
- WENDLER, G. (1964). Laufen und Stehen der Stabheuschrecke *Carausius morosus*: Sinnesborstenfelder in den Beingelenken als Glieder von Regelkreisen. *Z. vergl. Physiol.* **48**, 198-250.
- WENDLER, G. (1968). Ein Analogmodell der Beinbewegungen eines laufenden Insekts. In *Kybernetik 1968*, (eds H. Marko & G. Faeber), pp. 68-74. Muenchen-Wien: Oldenbourg.
- WENDLER, G. (1978). Kybernetik der Bewegung. In *Kybernetik 1977*, (eds G. Hauske & E. Butenandt), pp. 11-34. Muenchen-Wien: Oldenbourg.
- WILSON, D. M. (1965). Proprioceptive leg reflexes in cockroaches. *J. exp. Biol.* **43**, 397-409.
- WILSON, D. M. (1966). Insect walking. *Ann. Rev. Ent.* **11**, 103-122.
- WONG, R. K. S. & PEARSON, K. G. (1976). Properties of the trochanteral hair plate and its function in the control of walking in the cockroach. *J. exp. Biol.* **64**, 233-249.