PERTURBATION OF THE MOTOR SYSTEM IN FREELY WALKING COCKROACHES

I. REAR LEG AMPUTATION AND THE TIMING OF MOTOR ACTIVITY IN LEG MUSCLES

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Summary

1. The effects of amputation of a *rear* leg on the pattern of motor activity in the legs of freely walking cockroaches (*Periplaneta americana* L.) were studied.

2. Amputation affected both the frequency and the timing (phase) of motor bursts during a stepping cycle. Bursts in the stump of an amputated rear leg and in the contralateral (intact) rear leg often occurred at two or three times the frequency of bursts in the other legs. The remaining legs also showed multiple bursting during some steps.

3. Amputation affected the phase of motor bursts in two different ways. First, for every leg pair, phase was more variable after amputation, whether or not the mean phase was affected. Second, for some leg pairs, the mean phase itself was altered. During most steps, the timing of motor bursts in the stump of the amputated leg was walking-speed-dependent relative to bursts in the anterior legs. In contrast, the timing of bursts in the stump relative to bursts in the legs across the body from it showed no such speed-dependent timing. Timing between bursts in pairs of intact legs also showed either speed-dependent or speed-independent effects, depending on the pair under consideration.

4. The effects of amputation were not consistent. After loss of a leg, bursts in some leg pairs occurred synchronously in some insects and alternately in others. Even in single insects there were cases in which the timing between bursts in two legs switched from one value to another during walking.

5. These effects of amputation were manifest during slow walking only. At higher speeds, the timing of motor bursts in different pairs of legs was consistently closer to that seen during walking in intact insects.

6. Three conclusions are drawn from these results. (i) During slow walking, sensory feedback from the legs helps maintain the timing of adjacent ipsilateral leg pairs, but has little influence on contralateral pairs. (ii) During slow walking, either sensory input is quite variable, or it has variable effects on the motor pattern. (iii) During fast walking, sensory input from the legs seems to play a minimal role, if any, in the timing of the motor pattern of walking.

Introduction

For many decades, insect locomotion has been studied as a model for the generation of motor patterns (see Delcomyn, 1985a,b, Graham, 1985, for reviews). These studies have yielded important insights into motor organisation. For example, Wilson's (1961) early experiments with locust flight showed that the control system for this behaviour had a strong central component. This observation was later extended to other behaviour and other animals and generalised (Delcomyn, 1980). Later work by Wendler (1974) and by Pearson et al. (1983) showed that sensory feedback also played a critical role in the generation of each specific motor behaviour.

More recently, there has also been some interest in the study of motor systems for the practical role that knowledge of their organisation might play in the development of complex human prosthetic devices (Loeb, 1989) or of robotic walking vehicles. Insect walking has attracted attention as a possible model for a six-legged walking vehicle (Pearson and Franklin, 1984; Cruse, 1990).

In the case of both basic and applied research, a favourite approach to the study of walking has been to challenge the locomotor system with a perturbation and study its response in order to draw inferences about the rules by which it is organised. For example, experiments in which parts of the central nervous system are lesioned (Greene and Spirito, 1979; Dean, 1989), or inappropriate sensory feedback is provided through sense organ manipulation (Graham and Bässler, 1981; Bässler, 1979), have yielded insights into the role of central and peripheral coupling mechanisms in leg coordination.

Leg amputation is one of the oldest and most popular challenges to the walking control system. Amputation experiments were first reported by Carlet (1888), whose interest was in whether rendering the insect quadruped would change its mode of walking. As he noted, insects do show a dramatic change in gait when the two middle legs are amputated (see review by Delcomyn, 1985a). In current terms, this result is thought to be caused by loss of sensory input from the amputated legs. (The stumps that are left are too short to touch the walking surface.) The altered sensory input from the intact legs due to the changed mechanics of walking presumably also plays a role, although this role has not been defined clearly.

In spite of the long history of amputation experiments, physiologists have generally ignored the method, and the most recent studies involving amputation have only been behavioural (Hughes, 1957; Delcomyn, 1971b; Graham, 1977). These studies show a variety of effects of leg loss, but of course do not give any precise information about the motor pattern being delivered to the leg muscles. This is unfortunate, because knowledge of the pattern of motor activity in the stump and the intact legs after loss of a leg would provide additional clues to the organisation of the motor control system for walking and allow testing of current models of walking control. Furthermore, this knowledge would also provide valuable data on which to test recent hypotheses about the generation of multistable motor patterns and the mechanisms responsible for switching between them (Schöner and Kelso, 1988).

The purpose of the experiments described in this and the companion paper (Delcomyn, 1991) was to determine the effects of the amputation of a single rear or middle leg on the timing of leg motor activity in free-walking cockroaches. Front leg amputation was not studied because amputation is done at the trochanter, and the articulation and size of the coxa that is left allows the insect to continue to use the front legs. This therefore prevents some of the changed sensory input that it is the purpose of the experiments to produce. In addition to the specific effects of rear and middle leg amputation, the data suggest that, during slow walking, sensory input plays a strong role in the timing of motor bursts in ipsilateral pairs of legs and a much weaker role in the timing of bursts in contralateral pairs. It seems to play little role for any leg pair when the insect walks relatively fast.

Materials and methods
Recording of muscle activity

The experiments described in this paper were carried out on 26 male American cockroaches, *Periplaneta americana* L., taken from the colony in the Department of Entomology, University of Illinois at Urbana-Champaign. Insects were prepared and data were processed and analysed using standard methods (Delcomyn and Usherwood, 1973; Delcomyn, 1988, 1989). Briefly, records of electrical activity (electromyograms) of extensor (depressor) trochanteris muscles in the coxae of the legs of the insects were made while an insect walked freely in an enclosed arena. The muscles extend and depress the trochanter (hence the two synonymous names), and therefore the femur to which it is fused, providing most of the driving force of forward progression. (The exact contribution of each pair of legs to progression varies with the position of the legs, front, middle or rear.) The data on which this report is based came only from the steps of insects walking straight at steady speeds. Because recordings were not taken from every leg in some early experiments, the numbers of steps recorded from the legs is not the same for each. However, leg timing was examined for a particular leg pair in at least nine individuals, counting both right and left leg amputations.

Amputation of either a right or left rear leg was done under CO_2 anaesthesia or by a quick snip with scissors as the insect was standing still, at the fused joint between the trochanter and the femur. In the latter cases, insects showed no signs of distress. In a few insects it was necessary to cut through the trochanter, or even to remove the trochanter in order to keep the stump short enough so that it would not touch the ground. Recordings started as soon as the insect began to walk around the arena spontaneously, which generally occurred within a few minutes of the operation. A gentle touch with a fine brush was sometimes used to induce faster locomotion. Evoking extremely fast escape running was avoided because rapid leg movements yielded records with a high incidence of movement artefact and because they tended to reduce the quality of subsequent recordings even during slow walking. In order to have a consistent limit on which records of fast

walking were to be used, recordings were not used if they contained any fast axon activity in the muscles.

Data analysis

Electromyograms (EMGs) were filmed, and the starts and ends of bursts digitised and stored on disk in a personal computer, as described previously (Delcomyn, 1988). The digitised information on burst starts and ends was used to compute burst periods and the delay between the start of a burst in one leg and the start of a burst in another, or between the ends of bursts in different legs. The phase of the motor activity in one leg relative to that in another, a measure of the temporal relationship of motor activity in the two legs, was calculated from the delay and period data (phase=delay/period). Exact coincidence of delay and period was defined as a phase of 1.0. Phases were calculated based on periods defined from burst start to burst start or burst end to burst end, using the appropriate delay values. Phases for the figures in this paper are based on periods and delays calculated on burst ends. There was no significant difference between these values and those based on burst starts. In order to keep phase scattergrams to a manageable size, phases were not calculated for the handful of steps for which the step period was greater than 750 ms.

Calculations of phases were made for every pair of adjacent legs and every pair of legs that normally move together in the typical insect alternating triangle gait (Delcomyn, 1985a). Custom-made computer programs were then used to plot the phase values as a function of burst period (the reciprocal of walking speed) for each leg pair. Summary plots were also prepared in which data from a particular pair of legs were combined from all insects together. Individual and summary plots were used to examine individual variability and overall trends. Plots for the figures were generated on a graphics plotter by the program Sigma Plot (Jandel Scientific, Corte Madera, California). To avoid obscuring trends in the data in these plots by overburdening them with points, data in the summary plots in the figures were restricted to steps from sets of five insects each.

The custom-made programs that were used also computed a variety of statistical measures of data distribution. Circular statistical methods were used to assess the concentration, or sharpness, of the distributions, which is measured by the mean vector length. The greater the mean vector length (to a maximum of 1.0), the sharper is the distribution. Details of the statistical methods are given elsewhere (Delcomyn, 1989).

Results

Motor patterns in amputee insects

Each cockroach missing one of its rear legs walked without difficulty, but gave the impression that its walking was not quite as smooth as normal. One general effect of the amputation seemed to be to reduce the willingness of the insect to
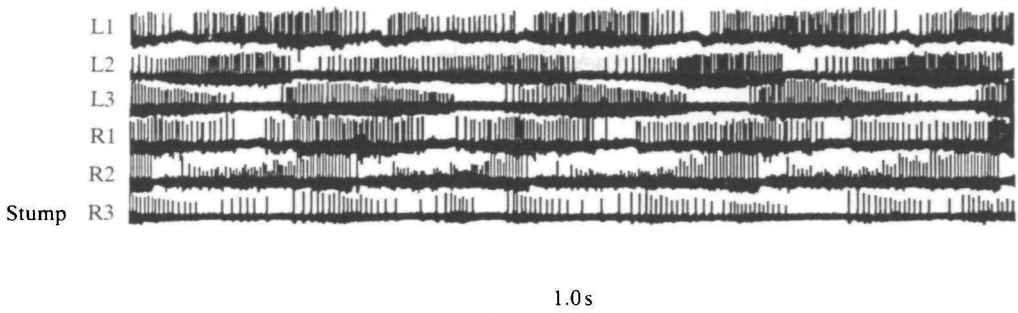
Fig. 1. Record of electrical activity in the extensor trochanteris muscles of each of the six legs of a freely walking cockroach from which the right rear leg has been amputated at the trochanter. Note the multiple bursting in the R3 record.

run. It was more difficult to induce walking at stepping rates above $14-15$ steps s^{-1} in amputee insects than in intact ones.

Examination of the pattern of motor activity in the leg muscles after amputation showed other effects (Fig. 1). One of these was the occurrence of multiple bursts in legs of amputee insects. Multiple bursting is the presence of several bursts of motor activity in a muscle in one leg during the same time period that muscles in other legs show only one. It has already been reported to occur in the stumps of amputated legs during walking (Delcomyn, 1988). Its occurrence there represents an expression of the locomotor control system rather than some other rhythmic behaviour (Delcomyn, 1988). As in 'normal' bursts during walking, activity in extensor muscles during multiple bursting reciprocated with activity in antagonistic flexor muscles. The movement of the stump itself was also double or triple the frequency of movement of the intact legs.

Examination of the motor activity in intact legs showed that in some cases these, too, showed multiple bursting (Fig. 2). Multiple bursts in intact legs most often occurred in the legs closest to the amputated one – the middle leg just in front of it,
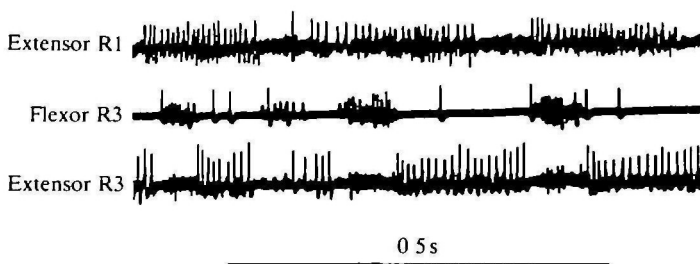

Fig. 2. Record of electrical activity in extensor and flexor muscles in the coxae of two intact legs during free walking. The left rear leg has been amputated at the trochanter. Note the presence of double bursts in both the extensor and flexor muscle activity in the intact rear leg relative to the intact front leg.

or the contralateral rear leg. Surprisingly, such bursts in an intact leg were not associated with an actual physical double or triple movement of the leg. Since walking insects were observed but not filmed, it is possible that some instances of a double movement could have occurred and not been seen, but it would have been impossible for all such occurrences to have gone unnoticed. A double step would be obvious in a slowly walking cockroach (only slowly walking insects showed double bursting). After the phenomenon had been noted in the motor record, several animals were kept under especially close observation during the entire period of recording. Double bursts were later found in the motor activity of at least one intact leg in these insects, yet no double steps, or any other kind of anomalous steps, were seen. Double bursting without overt double movement has also been reported in stick insects during walking on a treadwheel (Foth and Bässler, 1985a).

The timing of motor bursts

In all, phases for 13 leg pairs (26 counting reciprocal ones) were calculated. This included the timing of motor activity in every leg relative to its adjacent neighbours (seven leg pairs), and in every leg relative to non-adjacent legs of the same triangle (six leg pairs). [Intact cockroaches use their legs in sets of three (triangles), the front and rear legs on one side of the body and the middle leg on the other side moving together (Delcomyn, 1971a).] Both right and left rear leg amputee insects were studied. There was no apparent difference between symmetrical phases in the two types of amputees. That is, the distribution of phases between motor bursts in, for example, L1 and L2 when R2 was amputated was the same as the distribution of phases between bursts in R1 and R2 when L2 was amputated. Examples of phase distributions were selected for the figures so as to give illustrations from both right and left leg amputees.

The phase data for each of the 13 leg pairs are discussed in the following order: intact adjacent, intact non-adjacent (triangle) and amputee. Plots of phases from individual animals as well as summary plots from groups of five insects are shown. All comparisons to phase data for intact, freely walking cockroaches are to previously published data for such insects (Delcomyn, 1989).

Timing between one intact leg and another

Summary (Figs 3, 5, 7) and individual (Figs 4, 6, 8) plots for the phases of bursts in pairs of *intact* legs are shown in Figs 3–8. The plots are for bursts in ipsilateral (Figs 3 and 4) and contralateral leg pairs (Figs 5–8), and include every adjacent and triangle leg pair except pairs involving the amputated leg. Four features of these data were especially noteworthy.

(1) Amputation of a rear leg had an effect on the timing of motor bursts between every pair of legs, even those farthest from the amputated one. For the most distant legs, this effect manifested itself more as an increase in the scatter of the phase distribution than in any significant shift in its position or shape. This is illustrated in the distribution of phases for bursts between the middle and front legs contralateral (Fig. 3A) and ipsilateral (Fig. 3B) to the amputated one. The

mean phases between bursts in these two pairs of legs were 0.58 and 0.63, respectively, compared to a mean phase between middle and front legs in an intact insect of 0.53. The mean vector lengths (an inverse measure of scatter; see Materials and methods) for the phases of these two sets of legs, 0.83 and 0.75, respectively, indicated a greater scatter in these data than in the data from intact insects, in which the mean vector length for middle to front leg phases is 0.94. Phases between bursts in legs closer to the amputated one showed various other effects, as described below.

(2) Phases for intact contralateral leg pairs (R1/L1 and R2/L2) were considerably more scattered than phases for ipsilateral front/middle leg pairs. Whereas the mean vector lengths for the ipsilateral leg pairs were 0.75 and 0.83, respectively, as indicated above, that for one front leg relative to the other (Fig. 5A) was 0.63, and that for one middle leg relative to the other (Fig. 5B) was 0.43. It should be pointed out that, whereas most individuals showed phases between contralateral bursts that had the high scatter shown in the summary plots (Fig. 6A, triangles, 6B, circles), in some insects, the scatter of these values was not especially great (Fig. 6A, circles, 6B, triangles).

(3) The phases of bursts in non-adjacent (triangle) leg pairs showed a tendency to vary as a function of the speed at which the insect was walking. This is especially obvious in the distributions of phases for contralateral triangle leg pairs such as L2/R1 (Fig. 7). Intact, freely walking cockroaches show no significant shift in phase between any motor bursts during slow walking.

(4) For several pairs of legs, the timing of bursts in one leg relative to those in another was quite different from one insect to another, or even in one walking bout compared to another in a single insect. Put another way, the average phase between bursts in some leg pairs could not be predicted successfully in all cases following an amputation. In those leg pairs for which the overall scatter of the phases was not too great, this inconsistency of timing was manifested as a bimodal distribution in the summary plots (e.g. Fig. 3C at 0.7 and 0.4, Fig. 3D at 0.9 and 0.4, Fig. 7C at 0.9 and 0.2). The bimodality was due in part to different distributions of phases for different individual insects, but it was also due to a bimodal distribution of phase for steps from single insects. Examples can be seen in Figs 4D (triangles), 6B (circles) and 8A (circles).

Since bimodality appeared in the phase distributions for single insects, the different phase values must have been caused by variations in the mechanical or sensory situation from step to step during walking. They cannot have been due to unknown differences between individuals that seem to cause variation in the mean phase between motor bursts in different legs during free walking in intact cockroaches (Delcomyn and Cocatre-Zilgien, 1988).

Bimodal phase distributions were more common than cursory examination of the summary plots might suggest. For example, the distribution of phases for one of the animals represented in Fig. 4A (triangles) and one in Fig. 6B (circles) is bimodal, although the corresponding summary plots (Figs 3A and 5B, respectively) do not at first glance show much tendency to bimodality. Similar irregu-
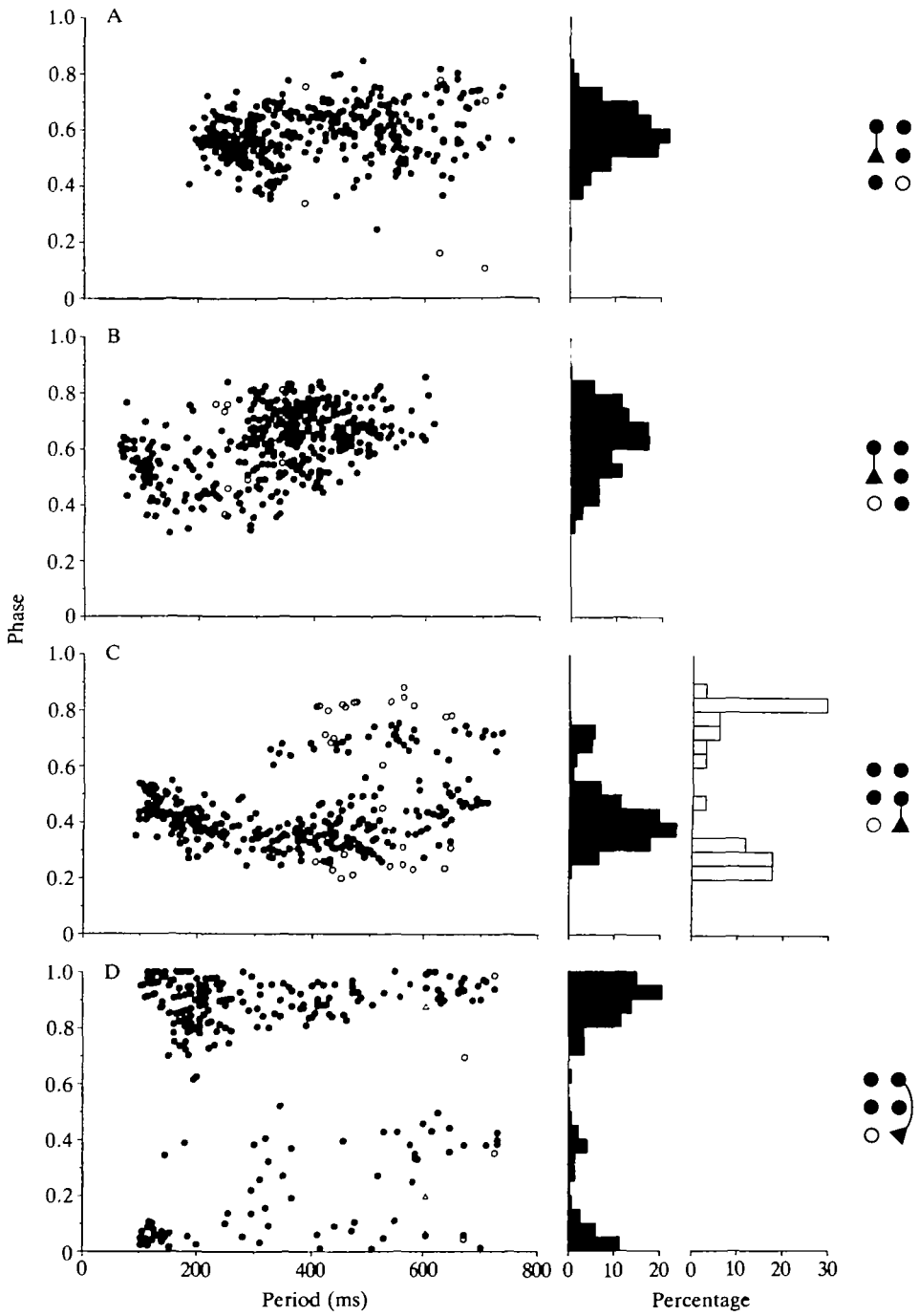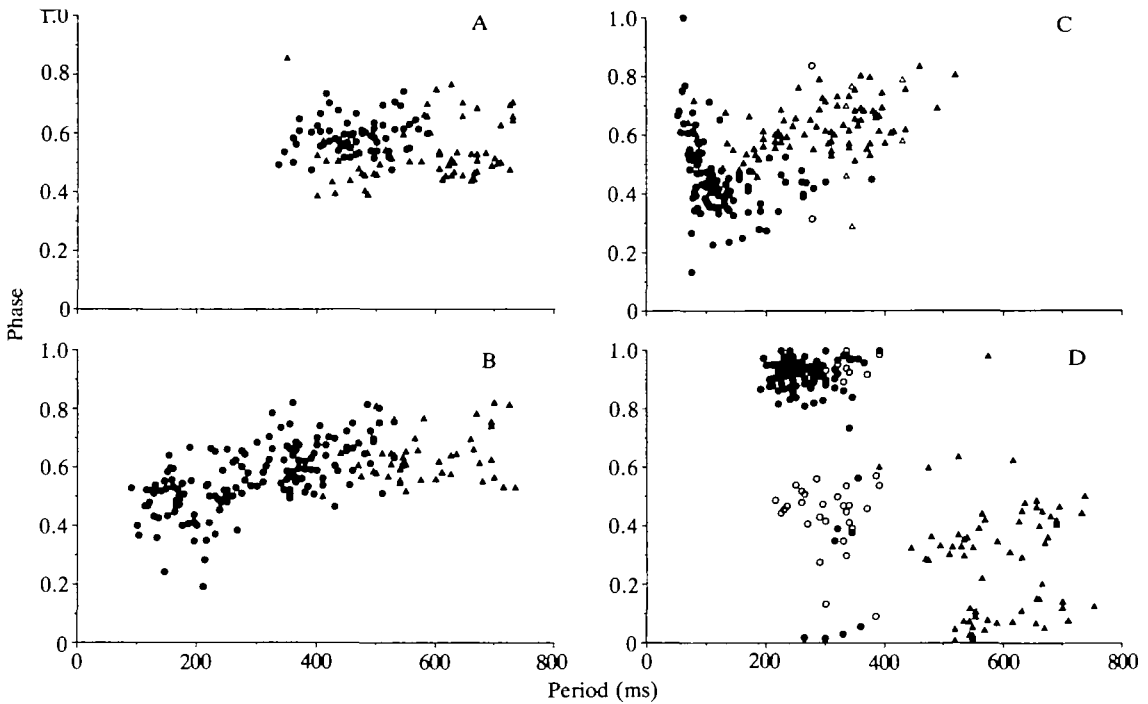
Fig. 3

Fig. 4. Plots of the phase of muscle activity in one intact leg relative to that in another intact, ipsilateral leg in individual insects. Circles represent a single insect, triangles (where present) another individual insect. Closed symbols, phases of single bursts; open symbols, phases of double bursts. Histograms are absent from these plots because most show data from two individual insects. Refer to the diagrams for the corresponding summary plot for a quick view of the logical relationship between the amputated, designated and reference legs. (A) Intact middle leg relative to front leg, contralateral to the amputated leg. Both animals: L3 amputated. (B) Intact middle leg relative to front leg, ipsilateral to the amputated leg. Both animals: R3 amputated. (C) Intact rear leg relative to middle leg, contralateral to the amputated leg. Both animals: R3 amputated. (D) Intact rear leg relative to front leg, contralateral to the amputated leg. Both animals: R3 amputated.

Fig. 3. Summary plots of the phase of muscle activity in one intact leg relative to that in another intact, ipsilateral leg. Data for five individual insects are combined in this and all subsequent summary plots (Figs 5, 7, 9). Scatterplots show the phases of bursts associated with individual steps as a function of burst period (the inverse of step frequency). All phases are calculated based on burst ends (see Materials and methods). ●, single bursts: O. double bursts (see text); △ (where they occur), triple bursts. Histograms give the distribution of the number of phases of different values (bin width 0.05 phase units), normalised as a percentage of the total number of phases. Filled histogram, distribution of phases of single bursts (●); open histogram (where present), distribution of double and triple bursts. Open histograms were drawn only if there were at least 20 multiple bursts present. The diagrams at the far right show the location of the amputated leg (open symbol; top is the front of the insect) and indicate the designated (triangle) and reference leg (connected to the triangle with a solid line) used in the phase calculations.
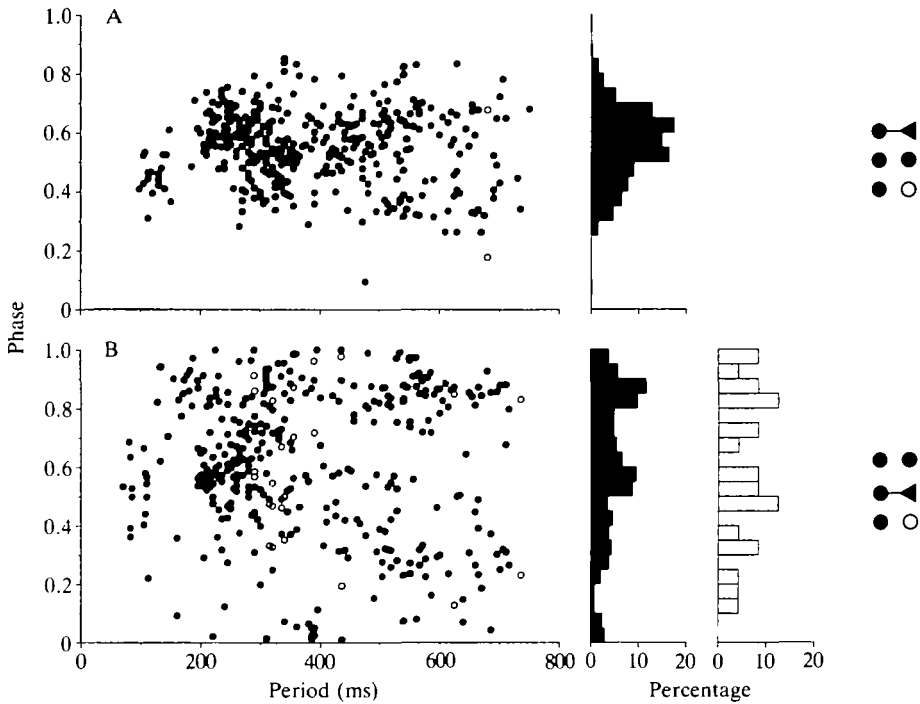
Fig. 5. Summary plots of the phase of muscle activity in one intact leg relative to that in its contralateral partner. Symbols as in Fig. 3.

larities in timing between legs in amputee insects that might have been due to bimodality in phase distributions have been reported behaviourally in freely walking stick insects (Graham, 1977).

Timing between amputee and intact legs

Summaries and selected individual plots of the distributions of phases of motor bursts in an amputated leg relative to those of an intact leg are shown in Figs 9 and 10. Perhaps the most striking feature of these summary plots was the obvious period-dependent distribution of phases of stump bursts relative to bursts in the leg in front (Fig. 9A) and the period-independent distribution of phases of stump bursts relative to bursts in the contralateral rear leg (Fig. 9B). This difference between ipsilateral and contralateral phases was also apparent when the stump/front leg phases (Fig. 9C) and the stump/contralateral middle leg phases (Fig. 9D) were considered.

Period-dependence of phasing was only seen in steps during which the stump gave single bursts relative to bursts in the intact legs. That is, there was no period-dependence of multiple bursts. The data do not allow a determination of whether this is due to a genuine difference in the behaviour of the timing of multiple *versus* single bursts, or whether it is a consequence of the paucity of multiple bursting a
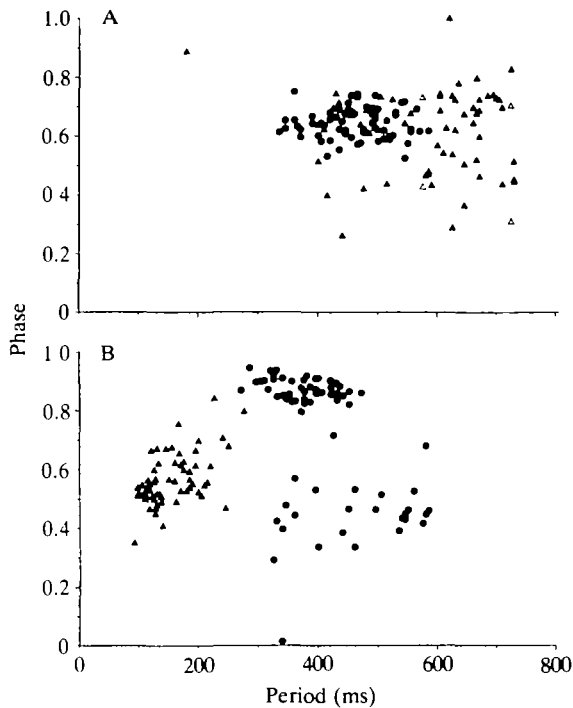
Fig. 6. Plots of the phase of muscle activity in one intact leg relative to that in its contralateral partner for individual insects. Symbols as in Fig. 4. (A) Intact front leg, ipsilateral to the amputated leg, relative to the contralateral front leg. Both animals: L3 amputated. (B) Intact middle leg, ipsilateral to the amputated leg, relative to the contralateral middle leg. Both animals: L3 amputated.

periods shorter than about 300 ms. (Period-dependent phase distributions are nearly flat at step periods greater than 300 ms.)

As was the case with phases between pairs of intact legs, phases between bursts in the stump and those in another leg were not always consistent or predictable. Most phases followed the trends described above. Some were clearly outside this pattern, however. For example, the insect whose L3/L2 phases (L3 amputated) are shown in Fig. 10A showed nearly complete period-independence for its single bursts. Nevertheless, phases for a few bursts fall into the low range shown in Fig. 9A.

The effects of walking speed on timing

In addition to the specific effect of the speed of progression on phases for certain leg pairs after amputation, there was also a general effect: essentially all the consequences of amputation described above were apparent only during relatively slow walking. That is, multiple bursting, period-dependent phase shifts and phase bimodality all occurred at walking speeds slower than about 5 steps s^{-1} (200 ms periods). They were rare or absent altogether during faster walking. In conse-
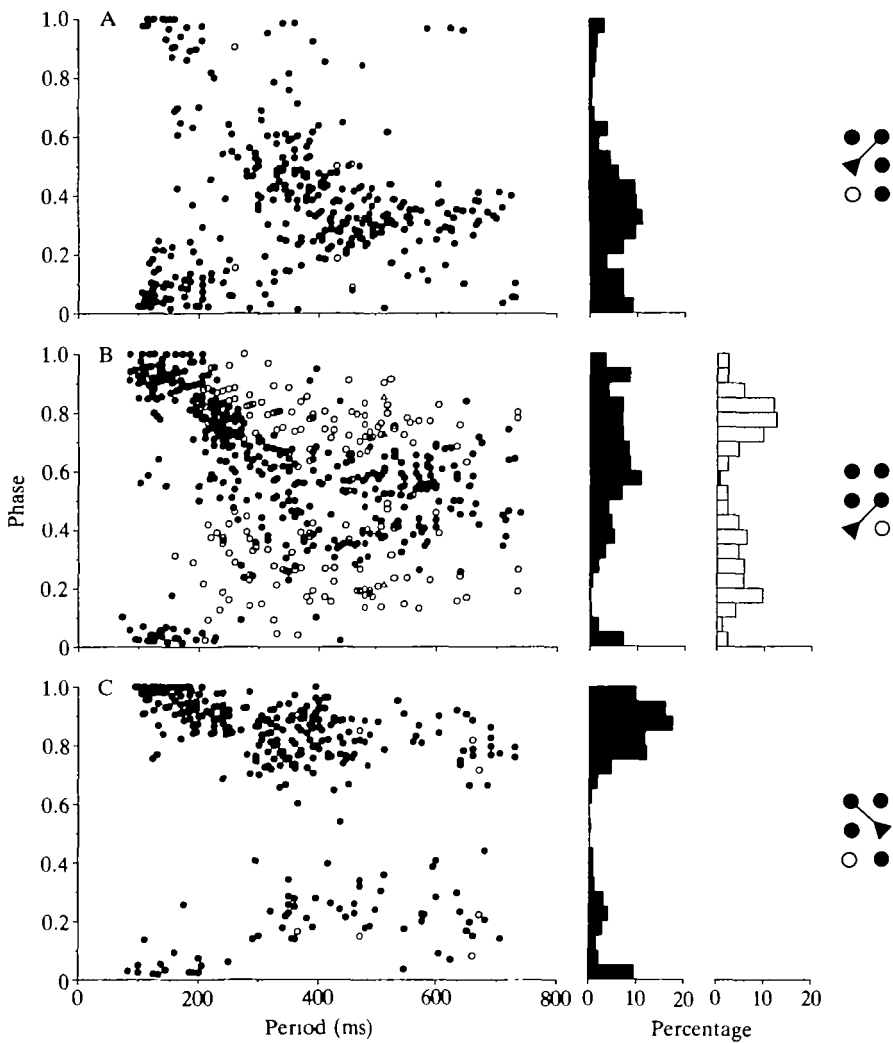
Fig. 7. Summary plots of the phase of muscle activity in one intact leg relative to that in an intact triangle leg (see Materials and methods). Symbols as in Fig. 3.

quence, if only steps taken faster than 5 steps s^{-1} were considered, the effects of amputation of a rear leg on the motor pattern in the stump and the intact legs were relatively minor. There was an increase in the scatter of timing between bursts, and there were some minor phase shifts between bursts in some leg pairs, but overall the motor pattern was not significantly disrupted.

Discussion

Amputation of one rear leg of a cockroach had several distinct effects on the motor pattern seen in extensor muscles in the legs during slow walking. It caused
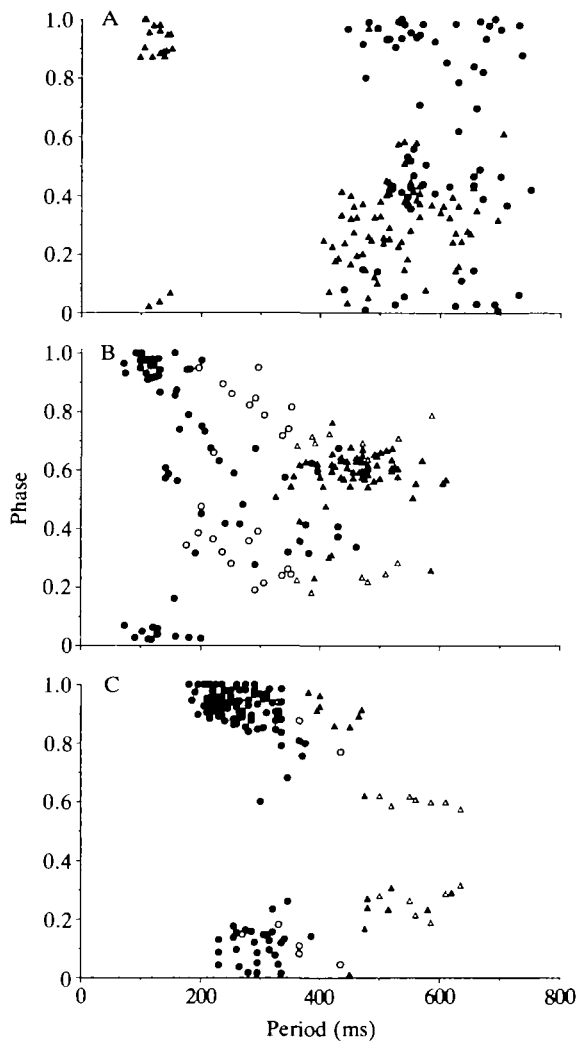
Fig. 8. Plots of the phase of muscle activity in one intact leg relative to that in an intact triangle leg for individual insects. Symbols as in Fig. 4. (A) Intact middle leg, ipsilateral to the amputated leg, relative to the contralateral front leg. Both animals: R3 amputated. (B) Intact rear leg, contralateral to the amputated one, relative to the contralateral middle one. Both animals: L3 amputated. (C) Intact middle leg, contralateral to the amputated leg, relative to the contralateral front leg. Both animals: R3 amputated.

multiple bursting in the stump and in adjacent intact legs, it caused disruption of timing between bursts in all leg pairs (most seriously for those that included the stump), and it brought on a walking-speed-dependent phasing for bursts in some leg pairs. Some of the phase effects were inconsistent, in the sense that the timing between bursts in a particular pair of legs could be quite different from one individual to the next, or even in a single insect from step to step. The effects were
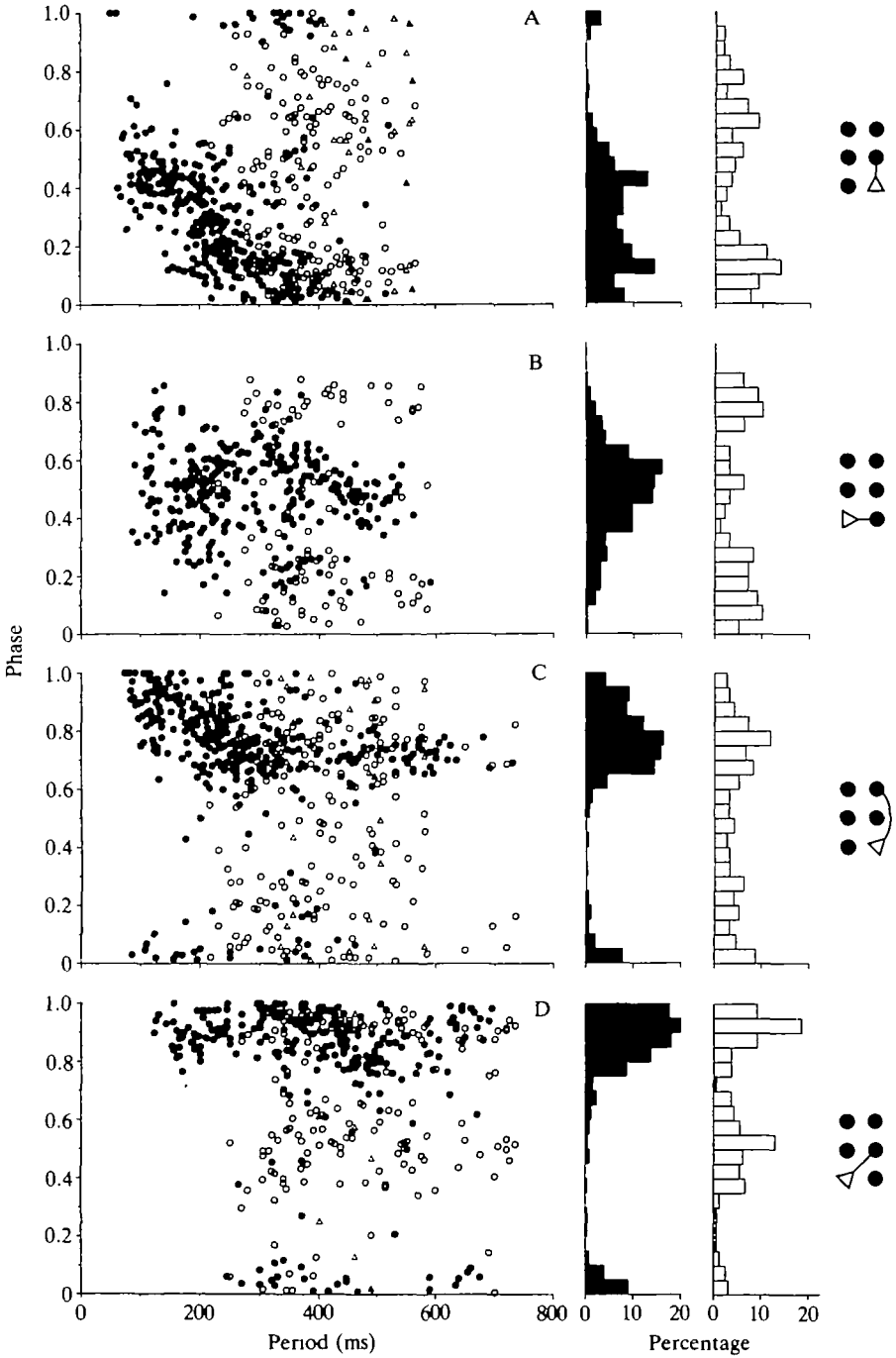
Fig. 9. Summary plots of the phase of muscle activity in the stump of an amputated leg relative to that in an adjacent intact leg and in an intact triangle leg. Symbols as in Fig. 3.
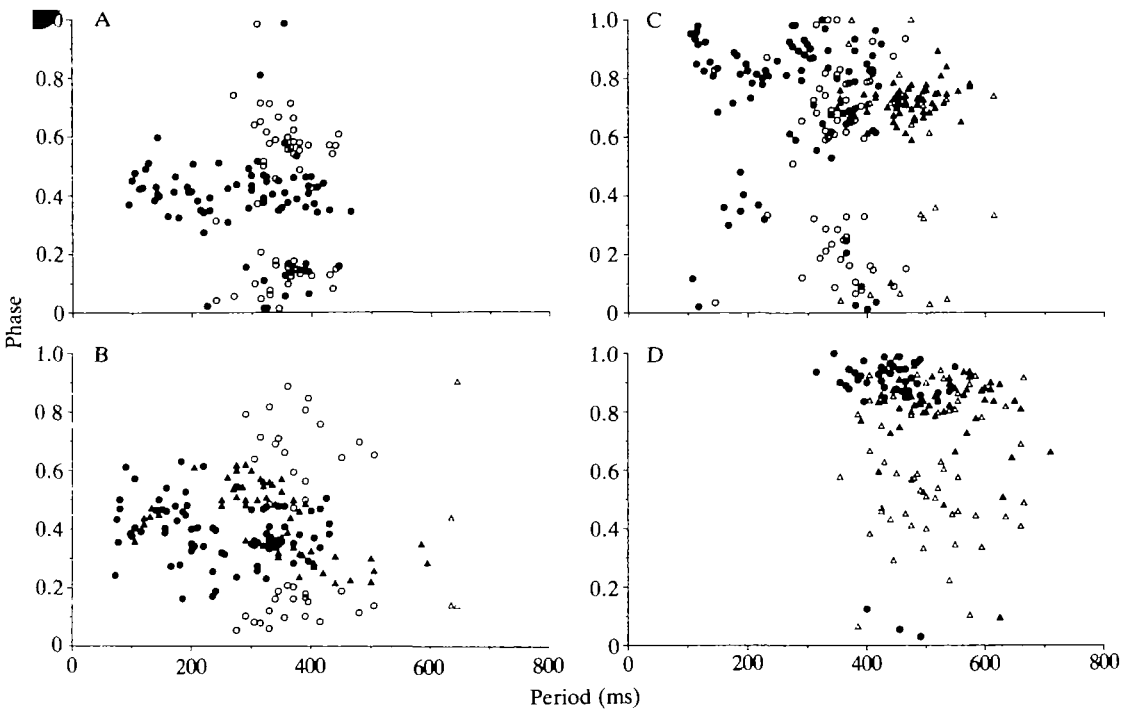
Fig. 10. Plots of the phase of muscle activity in the stump of an amputated leg relative to that in an adjacent intact leg and in an intact triangle leg for individual insects. Symbols as in Fig. 4. (A) Amputated rear leg relative to the ipsilateral middle leg. L3 amputated. (B) Amputated rear leg relative to the contralateral rear leg. Circles: R3 amputated; triangles: L3 amputated. (C) Amputated rear leg relative to the ipsilateral front leg. Both animals: L3 amputated. (D) Amputated rear leg relative to the contralateral middle leg. Both animals: R3 amputated.

apparent during slow walking only. These results will be discussed here in terms of the control of stepping in the cockroach. The broader implications for control of insect walking in general will be considered in the companion paper (Delcomyn, 1991).

Multiple bursting

Multiple bursting or multiple stepping have been demonstrated in various insects. Macmillan and Kien (1983) have shown that amputated stumps of legs in locusts will move at frequencies higher than those of the intact legs. Multiple stepping has been observed in intact legs of free-walking katydids (Graham, 1978) and of stick insects during walking on a pair of differentially loaded treadwheels (Foth and Graham, 1983) and on a treadwheel and a motor-driven belt (Foth and Bässler, 1985b). For some steps Foth and Bässler (1985b) reported multiple bursting without multiple stepping, as seen here. The phenomenon has also been reported in stick insect legs after the chordotonal receptor apodeme has been crossed (Graham and Bässler, 1981). Graham (1977) characterised the movements

of a rear leg stump during walking in a stick insect as erratic. This could have been' due to double or triple movements caused by multiple bursts. In cockroaches, multiple bursting has been described in deafferented animals by Pearson and Iles (1973), as well as in middle leg (Delcomyn, 1988, 1991) and rear leg (this paper) amputee insects.

In nearly all of these examples, multiple bursting or stepping is induced by an altered sensory input from one or more legs. This supports the view that sensory input helps keep the legs synchronised with one another. One way in which it might carry out this function is to help maintain the excitation of extensor muscles. In the stump of the amputated leg, shorter than normal burst durations (leading to more than one burst in what would otherwise be only a single cycle) could be due in part to the lack of input from the trochanteral campaniform sensilla (CS). Pearson (1972) showed that stimulation of these CS in the cockroach increased the firing of femoral extensor muscles in the coxa. Furthermore, when the insect was given a load to pull (which provided stronger stimulation to the CS), the duration of its extensor bursts increased. It seems likely that loss of CS input altogether would therefore allow the extensor motor neurones to fire in shorter, more frequent bursts. Multiple bursting in an intact leg could be caused by interaction of the network of neurones controlling the stump with those controlling the intact leg.

The effects of amputation on timing

That there should be an effect on the timing of motor bursts through loss of a rear leg in a freely walking cockroach is hardly surprising. What is surprising is the extent of the effect. The average phase changes reported here can only come about if there is a change in timing not only in the occurrence of bursts in the stump of the amputated leg relative to those in the intact legs, but also in the timing of bursts of each of the intact legs closest to the stump relative to those in the more distant ones, as well as an overall shift in timing between right and left legs. The extent of these shifts in timing was unexpected because loss of a single rear leg would not seem to impose great mechanical constraints on the insect. With only minor adjustments in leg placement (Hughes, 1957), the timing of the legs remaining after the loss of a single rear leg *could* remain nearly unaltered without severe loss of stability during walking, since there is still always one leg available for placement on the walking surface on the operated side even after the amputation.

The cause of the changes in timing that result from amputation is presumably the changed sensory condition that amputation brings about, especially the changed output from trochanteral campaniform sensilla. Removal of the tibia and tarsus has no significant effect on walking coordination (Delcomyn, 1971b), and fitting the insect with an artificial limb after removal of all of a leg distal to the trochanter restores the normal gait (F. Delcomyn, unpublished observations). Therefore, sense organs located more distally than the trochanter seem not to play the most critical role in determining motor burst timing.

The changed sensory condition has two components, the loss of input from the amputated leg and the altered input from sense organs in the intact legs. Both of

these may play a role. For example, in stick insects there is an interaction between two adjacent, ipsilateral legs such that one leg tends not to initiate its swing until the one behind it has taken up its supporting position (see review by Bässler, 1987). If this effect is due in part to the influence of the trochanteral CS, then eliminating the output from these sense organs will result in a delay in the initiation of a middle leg swing as it 'waits' for the signal from the rear leg CS that the missing leg has begun its stance. Additionally, the middle leg may wait because the rear leg does not take up its supporting position, leading to sustained stimulation of the middle leg CS. The feedback effect of this CS input on the middle leg's pattern generator would then be to delay swing (Pearson, 1972). In either case, the result will be a reduction of the phase of the motor bursts in the rear leg stump relative to bursts in the middle leg, as observed during slow walking.

The changed movement of the middle leg will, in turn, have a dual effect on the leg in front of it. The delay of its swing will delay the swing of the front leg because the CS in the middle leg will continue to signal ground contact later than they normally do. However, the CS in the middle leg will fire more strongly than usual at the beginning and end of the middle leg stance, because the rear leg is not there to take its normal share of the load. This increased firing at the beginning of middle leg stance may counter the delay of the swing of the front leg to some extent, causing the middle/front phase to increase (Fig. 3B). The timing of the front leg is nevertheless delayed relative to that of the rear leg stump, as shown by the decrease in the phase between the rear and front leg bursts (Fig. 9C).

The results also suggest that coupling mechanisms between pairs of ipsilateral legs do not have the same basis as coupling between contralateral pairs, and that the coupling is not equally strong. This is most obvious when one compares the timing of bursts in the stump relative to those in the middle leg in front of it to the timing of those bursts relative to those in the opposite rear leg. In the former case (Fig. 9A), timing is strongly influenced by the burst period (the reciprocal of the speed at which the insect is walking), whereas in the latter case it is not (Fig. 9B). Differences also occur in the timing for other ipsilateral leg pairs compared to that for contralateral ones (compare Fig. 3A,B,C with Fig. 5A,B). The considerably greater scatter for the timing of contralateral pair bursts suggests that coupling across the body might be weaker than that along one side. This conclusion is supported by observations on stick insects (Cruse and Knauth, 1989).

Inconsistent timing

The extraordinary variability, even inconsistency, in timing between bursts in certain pairs of legs described in this paper has not previously been noted explicitly. Graham (1977) called attention to behavioural variability in timing after amputation of a leg in stick insects, and Hughes (1957) and Delcomyn (1971*b*) noted that it occurred after amputation in cockroaches, but no mention was made of completely different timing for a single pair of legs in different insects or the same insect during different walking sequences. An examination of the data of

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Hughes (his Fig. 4) suggests that some of his insects might have shown different timing relationships between some legs, but he did not call special attention to it.

A complete description of which phases in certain leg pairs occurred together with other phases in other leg pairs would be required to determine if there are global rules about how the legs can move together. Such an analysis is under way, and preliminary results do suggest that certain phases tend to occur together, such that an insect may adopt an entirely different gait during some sequences of walking than during others. Phase inconsistency occurs in response to a middle leg amputation as well (Delcomyn, 1991), and there, too, the data indicate that distinctly different gaits may be used by the same insect during different walking sequences. In the context of Schöner and Kelso's (1988) concept of multistable motor pattern-generating systems, inconsistent or multimodal phase distributions might be the result of fluctuations in the input from the intact legs due to the altered loading that they experience.

Feedback and fast walking

In the cockroach, it has been suggested that, at high speeds of walking, some kind of centrally organised timing system (central pattern generator, or CPG) must exist, because known reflexes seemed to be too slow to be of value under these conditions (Zill, 1985). If sensory feedback plays a reduced role in timing during fast walking, then one would expect that any effects of amputation that are the result of an altered sensory input ought to be less apparent during such fast walking. This is just what one sees. All the major effects of rear leg amputation, multiple bursting, phase shifts and variable (inconsistent) phase relationships, disappear or are strongly reduced during fast walking. The result is that, at medium and high speeds of walking, timing between motor bursts in different legs is closer to that seen in intact insects than it is at low speeds of walking. Similar effects are observed after a middle leg amputation (Delcomyn, 1991). These results, which are quite different from those from the more slowly walking insects, strengthen the view that the cockroach has a neural control system specialised for fast walking.

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