

PERTURBATION OF THE MOTOR SYSTEM IN FREELY WALKING COCKROACHES

II. THE TIMING OF MOTOR ACTIVITY IN LEG MUSCLES AFTER AMPUTATION OF A MIDDLE LEG

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Summary

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

2. The general effects of amputating a middle leg are similar to those arising from amputation of a rear leg. These effects are: multiple bursting, more variable and inconsistent timing (phase) between bursts and a tendency for timing effects to appear only during relatively slow walking.

3. The phase of bursts in the amputated stump relative to bursts in the leg in front of it was speed-dependent. However, the phase of stump bursts relative to bursts in the legs across from and behind the stump were not especially dependent on the speed of walking. In general, the phases of bursts in most leg pairs seemed relatively little affected by the amputation except for an increase in scatter.

4. It is concluded that loss of a middle leg disrupts the motor pattern less severely than does loss of a rear leg. The implications of this and other results for the understanding of motor control are discussed.

Introduction

Amputation of both middle legs is a well-established technique for challenging the motor system of a walking insect (see review by Delcomyn, 1985). The technique has attracted special attention because of the dramatic gait change that results from it and because the effects can be reversed by providing artificial legs for the insect. The basis of the altered gait is thought to be loss of specific sensory input from the missing legs, since the segments that remain are too short to touch the ground. Restoration of the normal gait is considered to be due to restoration of this input *via* the artificial limbs.

Although the behavioural results of amputation experiments have been studied in several insects and in some detail (see review by Delcomyn, 1985), the effects of amputation on the pattern of motor activity delivered to leg muscles have not been

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thoroughly considered. This is unfortunate, since examination of the motor pattern after amputation may help verify the purported role played by sensory input in the gait change.

Amputation of a single rear leg has been shown in the companion paper (Delcomyn, 1991) to have effects on the timing (phase) of motor bursts in each leg relative to every other leg, although the effects are weaker in leg pairs that are farthest from the amputated one. Loss of a rear leg, however, does not present as severe a mechanical disturbance to walking as does loss of a middle leg, since the insect can still move the remaining five legs in the usual pattern without loss of balance. One might therefore expect that the effects of amputation of a single middle leg would be more dramatic.

The purpose of the experiments described in the present paper was to investigate the effects of amputation of one middle leg on the timing of motor patterns in the legs of freely walking cockroaches. The results suggest that, contrary to expectation, the effects of such an amputation are less severe than are the effects of rear leg amputation. The implications of this finding for locomotor control in insects is discussed.

Materials and methods

The experiments described in this paper were carried out on 31 male American cockroaches, *Periplaneta americana* L., taken from the colony in the Department of Entomology, University of Illinois at Urbana-Champaign. Experimental methods and analytical techniques used were the same as those described in the companion paper (Delcomyn, 1991) for the study of rear leg amputation, except that right or left middle legs were amputated.

Results

The pattern of motor activity

Cockroaches missing a middle leg walked quite well, although, as with those missing a rear leg, it is obvious that the gait was not entirely normal. (All statements regarding the effects of rear leg amputation refer to the companion paper: Delcomyn, 1991.) The pattern of motor activity in the coxae of all the legs of freely walking insects reflected this disturbed gait (Fig. 1), frequently showing significant differences in the timing between bursts in some leg pairs, and somewhat greater variability in the timing between bursts in others.

Loss of a middle leg resulted in multiple bursts (Delcomyn, 1988) in the stump of the leg. These multiple bursts in middle leg stumps were similar in appearance and general characteristics to those in rear leg stumps, as described previously.

Multiple bursts also occurred in the motor activity in intact legs. However, occurrence of multiple bursts in these legs was not especially common. Even the intact leg on the opposite side of the body from the amputated one did not show a great number of multiple bursts.

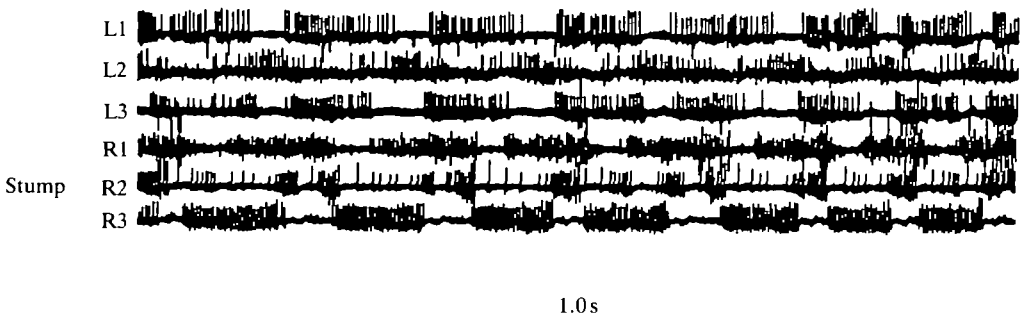


Fig. 1. Record of electromyographic activity in the extensor trochanteris muscles in the coxae of each of the legs of a freely moving cockroach after the removal of the middle right leg. Note the multiple bursting in the R2 record.

The timing of motor bursts

Phase, a measure of timing between two sets of bursts, was calculated for every leg relative to its adjacent neighbour (seven pairs) and relative to non-adjacent, triangle legs (six pairs; see Delcomyn, 1991, for a definition). Both right and left middle leg amputees were studied. There was no obvious difference between symmetrical phases (phases for mirror-image leg pairs for a right and left leg amputation) in the two types of amputees. For a few leg pairs there were minor differences in the exact shape of the phase distribution between timing plots for right *vs* left leg amputees, but these were not large enough to be considered indicative of a genuine right/left asymmetry. 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 order: intact adjacent, intact non-adjacent (triangle) and amputee. Plots of phases from individual animals as well as summary plots of data from five insects together (see Materials and methods, Delcomyn, 1991) are shown. All comparisons with phase data for intact freely walking cockroaches are with previously published data for such insects (Delcomyn, 1989).

Timing between one intact leg and another

Summary (Figs 2, 4) and individual (Figs 3, 5) plots for the phases of bursts in pairs of intact legs are shown in Figs 2–5. The plots show phases for bursts in ipsilateral (Figs 2 and 3) and contralateral leg pairs (Figs 4 and 5), and include every adjacent and non-adjacent (triangle) leg pair except those involving the amputated leg, which are discussed in the next section. Three features of the data deserve comment.

(1) The scatter of the timing between bursts in every pair of intact legs was increased by amputation of a middle leg. A similar effect is observed after loss of a rear leg. The increase in scatter is apparent in both summary plots (Figs 2, 4) and

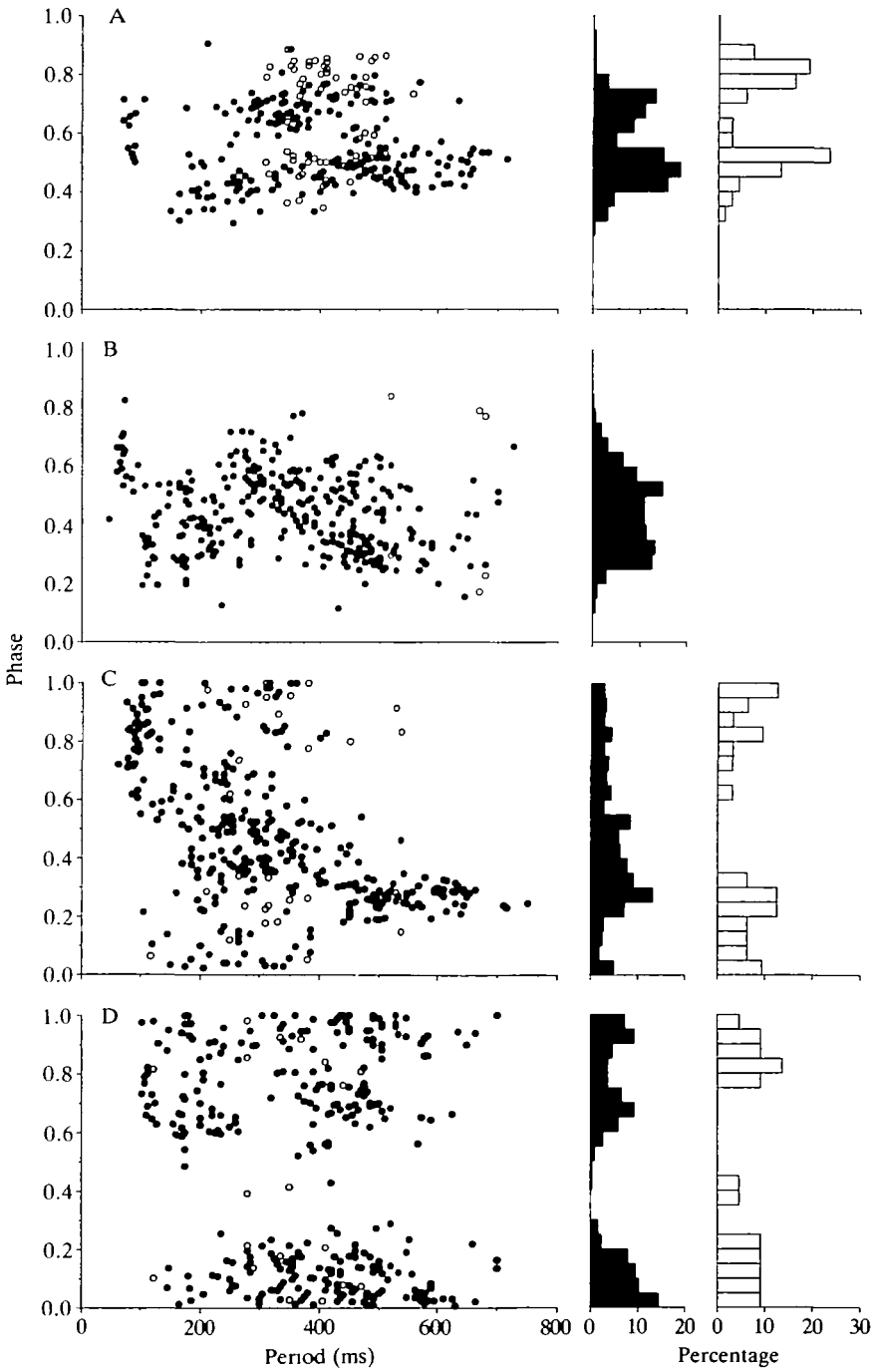


Fig. 2

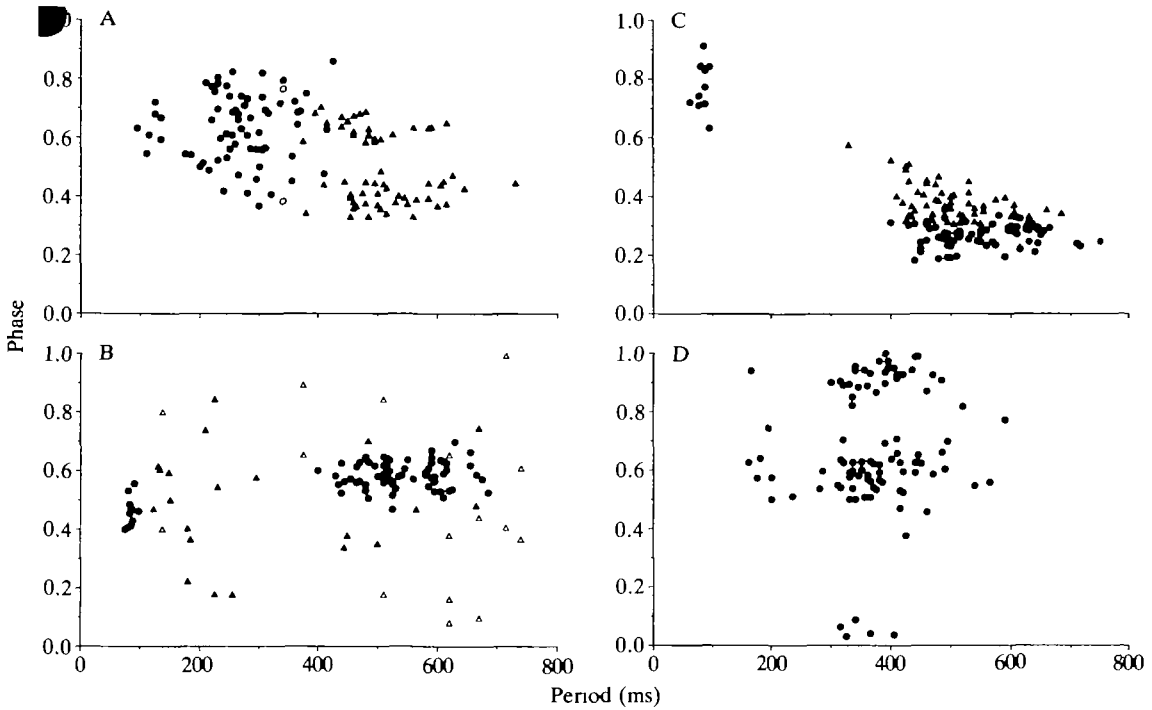


Fig. 3. Individual plots of the phases of bursts in one intact leg relative to bursts in another intact, ipsilateral leg. Closed symbols, phases of single bursts from individual steps. Circles and triangles represent data from different individual insects. Open symbols, phases from double bursts. (A) Intact middle leg relative to the ipsilateral front leg. Circles, L2 amputated; triangles, R2 amputated. (B) Intact middle leg relative to the ipsilateral rear leg. Both animals: L2 amputated. (C) Front leg relative to rear leg, ipsilateral to the amputated leg. Circles, L2 amputated; triangles, R2 amputated. (D) Front leg relative to rear leg, contralateral to the amputated leg. L2 amputated.

Fig. 2. Summary plots of the phases of bursts in non-amputee ipsilateral leg pairs relative to one another. Data for five individual insects are combined in this and all subsequent summary plots (Figs 4, 6, 8). Scatterplots show the phases of bursts associated with individual steps, as a function of burst period (the inverse of step frequency). Phase is computed for each burst relative to the cycle of bursting in the reference leg. The cycle is defined from the end of one burst to the end of the next. Symbols: ●, single bursts; ○, double bursts (see text); △ (where they occur), triple bursts. The histograms at the right of each phase plot show the frequency distribution of phases independent of burst period (bin width 0.05 phase units), normalised as a percentage of the total number of phases. Filled histograms, distribution of phases for single bursts (●). Open histograms (where present), distribution of phases for double and triple bursts (○, △). Open histograms are 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.

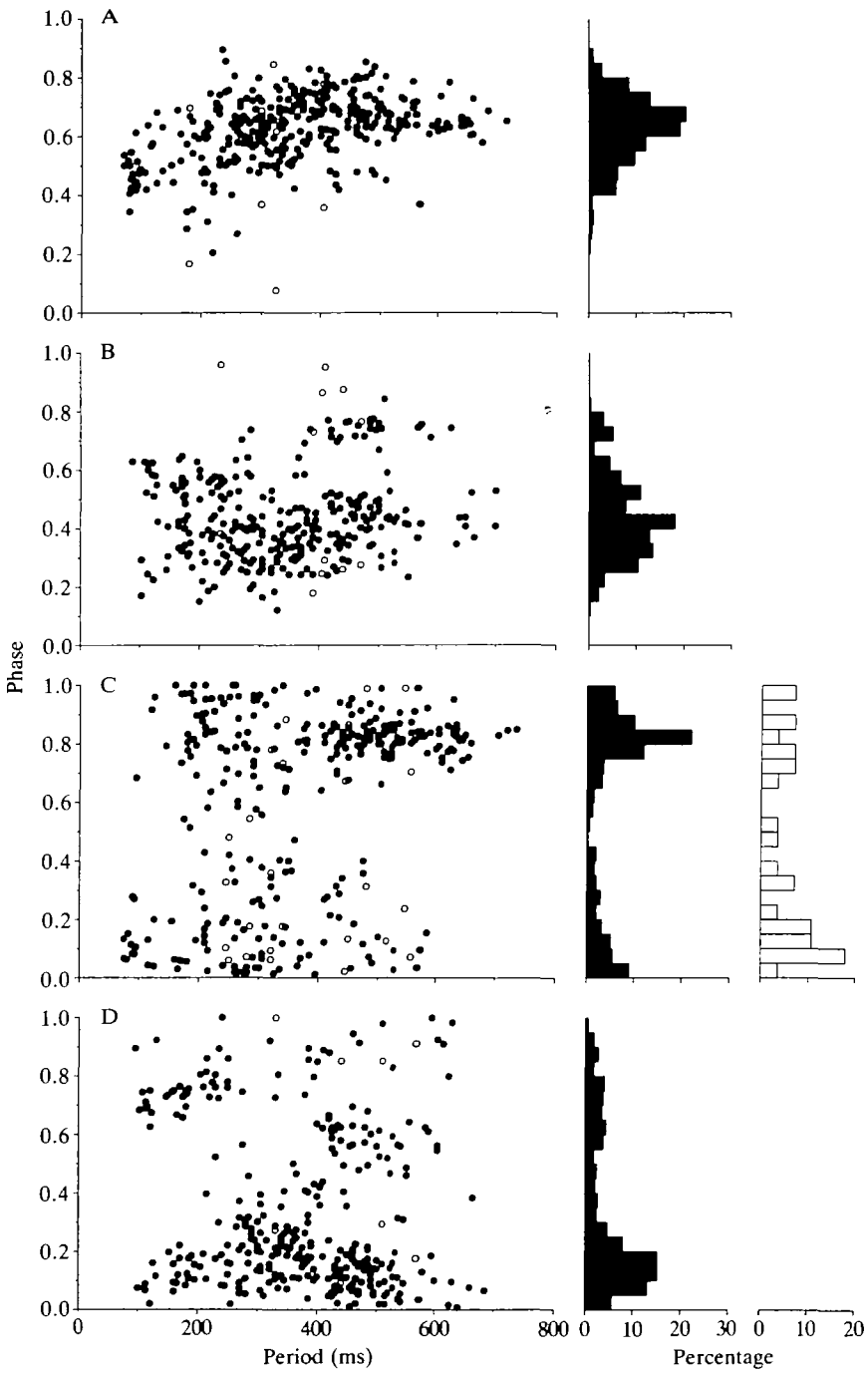


Fig. 4. Summary plots of the phases of bursts in one intact leg relative to that in its contralateral partner. Histograms and symbols as in Fig. 2.

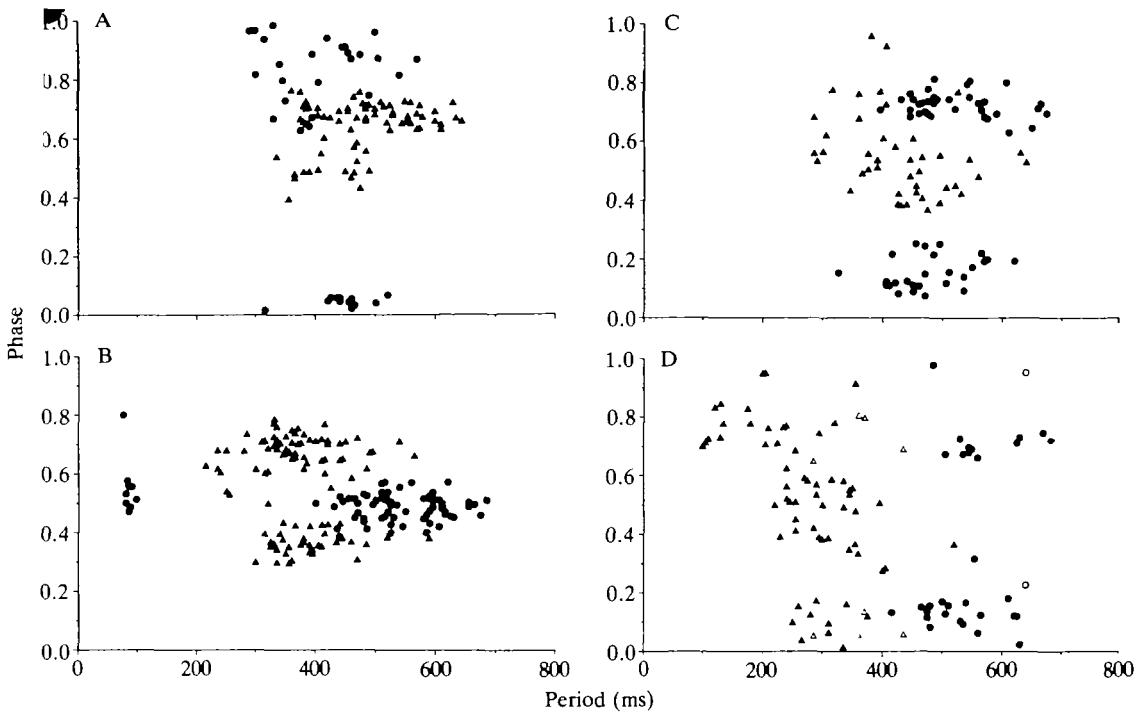


Fig. 5. Individual plots of the phases of bursts in one intact leg relative to that in its contralateral partner. Symbols as in Fig. 3. (A) Intact front leg (ipsilateral to the amputated one) relative to contralateral front leg. Both animals: R2 amputated. (B) Intact rear leg (ipsilateral to the amputated one) relative to contralateral rear leg. Both animals: L2 amputated. (C) Intact middle leg relative to contralateral front leg. Both animals: R2 amputated. (D) Intact middle leg relative to contralateral rear leg. Both animals: L2 amputated.

in phase distributions for individual insects. However, there can be considerable differences between individuals. For example, the phases of bursts in an intact middle leg relative to those in the rear leg behind it in the two insects shown in Fig. 3B show that the phases for one have a considerably tighter distribution (circles) than do the phases for the other (triangles).

(2) The second feature is, in a sense, a non-feature. In intact freely walking insects, the mean phase of a burst in one leg relative to a burst in an adjacent leg is about 0.5, whereas the mean phase of bursts in triangle legs relative to one another is about 1.0. Furthermore, at walking speeds of about 10 steps s^{-1} and slower (burst periods of 100 ms or greater), there is no change in the average phase as a function of burst period. Motor bursts in intact leg pairs of insects missing one middle leg generally exhibited similar characteristics. The mean phase for most leg pairs was not too different from the mean phase for those legs in intact insects (e.g. Figs 2B, 4B), and burst phases were independent of burst period (Figs 2A, B, 4). The major exception to the general case was the timing of bursts in a front leg

relative to those in the rear leg, for the legs that bracketed the amputated middle leg (Fig. 2C, discussed below). In this case, phase varied inversely with the burst period. The general result contrasted sharply with the results of rear leg amputation, which caused period-dependent timing and altered mean phases in several different pairs of intact legs.

(3) One of the most striking effects of middle leg amputation was the appearance of bimodal distributions of phases (e.g. Fig. 2A at 0.5 and 0.7; Fig. 2D at 0.0/1.0 and 0.7; Fig. 4D at 0.1 and 0.7). The scatterplots clearly showed that the two modes represented different phases produced at a single burst period for different steps. Plots of phases for individual insects showed that the two modes could be generated by the different steps of a single insect (Figs 3D, 5D). Sometimes one insect adopted one timing relationship between bursts in a particular pair of legs and another insect adopted another (Fig. 5C). In some cases the timing of bursts for one pair of legs in two individual insects was quite different (Fig. 5A), although the summary plot was too scattered to allow the ready identification of two different modes. Every individual did not necessarily show a bimodal phase distribution for a particular pair of legs (Fig. 5B, circles).

Bimodal phase distributions were also found after amputation in a rear leg, and irregularities in gait that may represent a similar phenomenon have been described in stick insects (Graham, 1977).

Timing between amputee and intact legs

Summary (Figs 6, 8) and individual (Figs 7, 9) plots for the phases of bursts in the amputated stump relative to those in the intact legs are shown in Figs 6–9. The plots include every adjacent (Figs 6 and 7) and non-adjacent (triangle) (Figs 8 and 9) leg pair involving the amputated leg.

Even cursory examination of the plots of phases involving motor bursts in the stump brought home the extent of double bursting as well as the enormous degree of scatter in these data. This is especially apparent from an examination of the histograms of phase frequencies shown at the right of each phase scattergram. Closer examination revealed other features. For all but one pair of legs, there was no apparent relationship between the average phase value at a particular burst period and the duration of the period. That is, phase in these cases was independent of burst period (Figs 6B,C; 8A,B). The exception was the distribution of timing between bursts in a stump and those in the ipsilateral leg in front of it, which was strikingly period-dependent (Fig. 6A). Middle leg amputees thus showed the same period dependence between bursts in the stump and those in the leg in front of it as did rear leg amputees. They also showed the period independence between stump bursts and those in the contralateral leg. The difference was that, in the case of the middle leg amputees, the period dependence with the ipsilateral front leg was the *only* period-dependent relationship that bursts in the stump exhibited.

Study of timing between stump and intact leg bursts in individual insects showed great individual differences in the response of the insect to an amputation. One,

insect showed nearly all multiple bursts with much scatter (Fig. 7B), whereas another showed no multiple bursts, and relatively little scatter of individual phases (Fig. 9A). Such great differences between individuals have been observed in rear leg amputees and, on a different level, even in intact insects (Delcomyn and Cocatre-Zilgien, 1988). A rather subtle trend in individual insects, that was nevertheless consistent in the data, was the tendency for phases between bursts in the middle leg stump and those in the ipsilateral or contralateral front leg to have tighter distributions (distributions with less scatter) than phases between a middle leg stump and the ipsilateral or contralateral rear legs in individual insects. This trend is well illustrated in Fig. 7, which shows the phase of multiple bursts from an amputated middle leg relative to the ipsilateral front (Fig. 7A) and rear (Fig. 7C) legs. These figures, from the same insect, are typical.

Other effects

The major effects described above, multiple bursting, period-dependent phase shifts and phase bimodality, occurred only during relatively slow walking. During walking faster than about 5 steps s^{-1} (burst period of 200 ms), not only were these effects absent but the average phase for bursts in any two pairs of legs (including the stump) tended to be close to that found in intact insects for that leg pair. This was apparent in many of the summary plots (Figs 2A, 4A, 6A) as well as in plots from individual insects (Figs 3C, 5B). This phenomenon was also present in rear leg amputees.

Discussion

Amputation of one middle leg of a cockroach had several distinct effects on the motor pattern seen in extensor muscles in the legs during slow walking. It caused multiple bursting in the stump and in adjacent intact legs and it caused mild disturbance of timing between some bursts and more serious disruption of timing between others. 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 apparent during slow walking only.

Three general effects, multiple bursting, inconsistent timing and the disappearance of these effects during fast walking, were also manifested after the amputation of a *rear* leg. The finding that these effects were not a specific response to the loss of a rear leg, but are apparently general responses to the loss of any leg, supports the view that they are manifestations of general organisational properties of the cockroach locomotor control system, as discussed in the companion paper (Delcomyn, 1991).

The effects of amputation on timing

The specific effects of middle leg amputation merit more detailed discussion because it is important to know what differences there might be between the

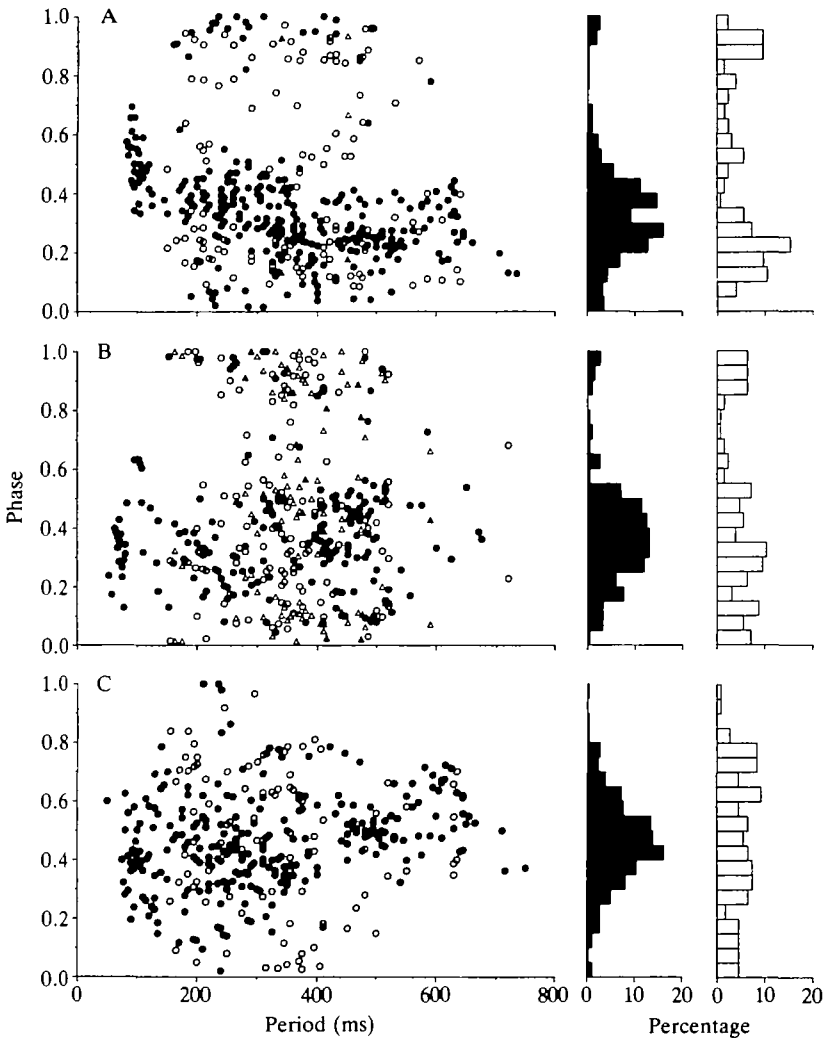


Fig. 6. Summary plots of the phases of bursts in the stump of the amputated leg relative to bursts in adjacent non-amputated ones. Histograms and symbols as in Fig. 2.

effects of the loss of legs that occupy different positions in the normal triangle of support. One might predict that loss of a middle leg would be a greater mechanical challenge to the insect, since the middle leg provides the only support on one side of the body when the front and rear legs on that side are lifted in their swing phase. One might also predict, as a consequence, that the effects of middle leg loss on the motor pattern would be greater than the effects of rear leg loss.

In many respects, the effects on timing of amputating a middle leg are similar to those that follow amputation of a rear leg. There is a strong period-dependent shift of phase between motor bursts in the stump and bursts in the leg in front of the

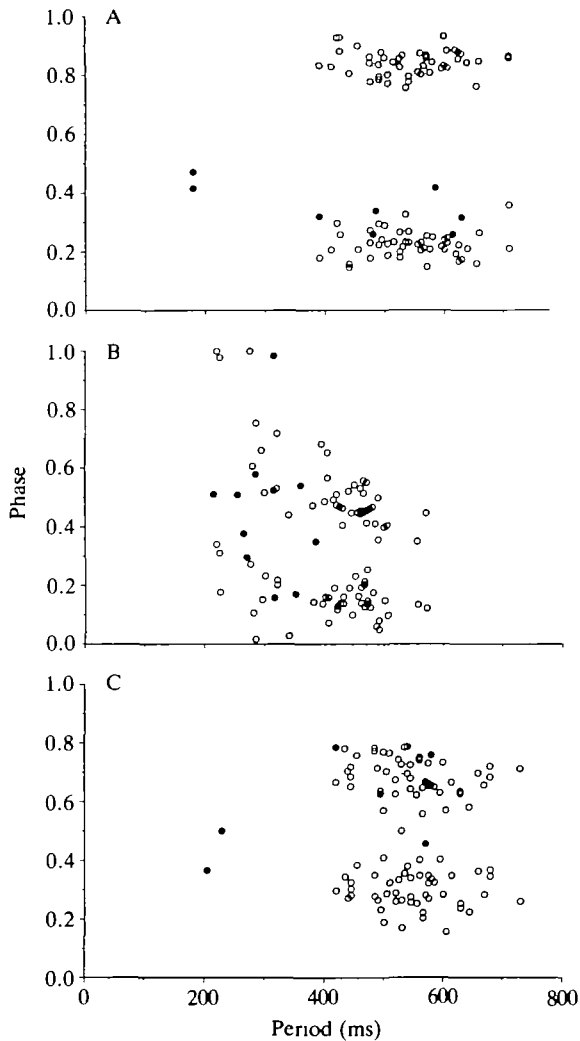


Fig. 7. Individual plots of the phases of bursts in the stump of the amputated leg relative to bursts in adjacent non-amputated ones. (A) Amputated leg relative to ipsilateral front leg. R2 amputated. (B) Amputated leg relative to contralateral middle leg. L2 amputated. (C) Amputated middle leg relative to ipsilateral rear leg. R2 amputated.

stump (Fig. 6A), but no such shift relative to bursts in the contralateral middle leg (Fig. 6B). This similarity of effects suggests that there may be a similarity in the coordinating pathways present between the middle and front legs and those present between the rear and middle ones. Furthermore, the difference between the effect of the amputation on ipsilateral compared with contralateral leg coupling also seems to be a general phenomenon, whichever leg is amputated.

In other respects, however, the amputation of a middle leg did not have the same effect on the timing of the motor pattern as loss of a rear leg did, and

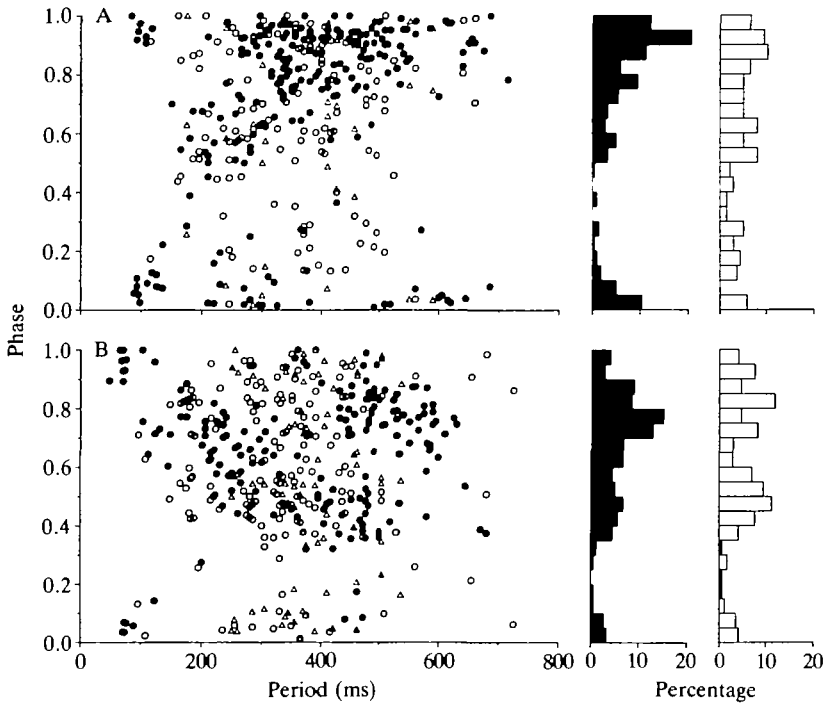


Fig. 8. Summary plots of the phases of bursts in the stump of the amputated leg relative to bursts in triangle non-amputated ones. Histograms and symbols as in Fig. 2.

certainly had a smaller effect than might have been expected. The presence of bimodal phase distributions, period-dependent phase shifts and scatter makes comparisons between phase distributions for rear and middle leg amputees difficult. Estimates of the average phase values between bursts in different legs at 400 ms periods for middle and rear leg amputees, however, suggest that fewer adjustments of the standard tripod gait are required to produce the timing seen in middle leg amputees than to produce that seen in rear leg amputees. At the very least, the phase distributions in the former case are certainly no 'worse' in the sense of being more scattered or more deviant from normal than the distributions following rear leg amputation. The prediction that timing ought to be more disrupted after loss of a middle leg than after loss of a rear one has, therefore, not been borne out.

Locomotor control

The contemporary view of locomotor (walking) control in insects has largely been shaped by recent work on the stick insect. In this insect, groups of neurones acting as relaxation oscillators require a variety of excitatory and inhibitory signals from the legs so that during stepping the transitions from stance to swing phase and *vice versa* may be made properly (see reviews by Bässler, 1987; Cruse, 1990). Earlier work on cockroaches had suggested that in these insects there was an

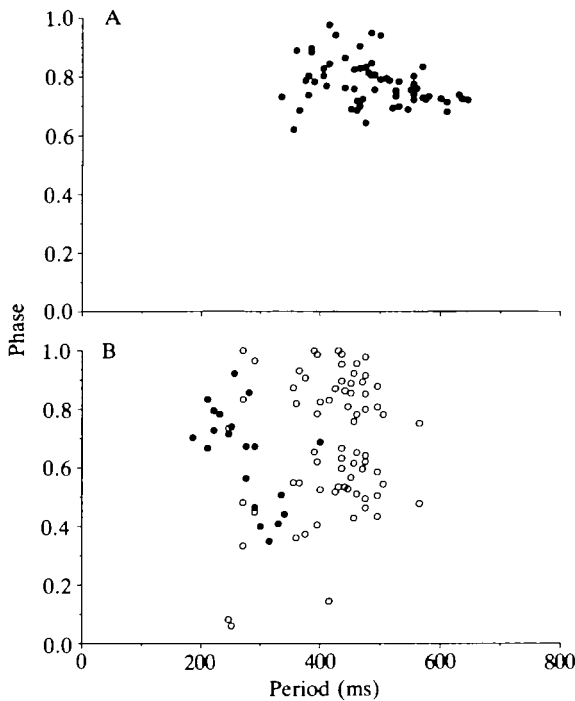


Fig. 9. Individual plots of the phases of bursts in the stump of the amputated leg relative to bursts in triangle non-amputated ones. (A) Amputated leg relative to contralateral front leg. R2 amputated. (B) Amputated leg relative to contralateral rear leg. L2 amputated.

intrinsic rhythm-generating network of neurones (a 'central pattern generator' or CPG) (Pearson and Iles, 1970; see reviews by Delcomyn, 1985; Graham, 1985). However, this notion has fallen out of favour to some extent, partly because of rethinking about what constitutes a CPG (Pearson, 1985) and partly because evidence for it in stick insects is lacking (Bässler and Wegner, 1983).

In this context, the results described in this and the companion paper contribute to our understanding of the control of walking in insects. First, the work strengthens the suggestion by Zill (1985) that, during medium to fast walking, cockroaches use central pathways for the main timing signals for motor bursts in different legs rather than relying on sensory feedback signals from the moving legs. For some insects, therefore, an understanding of the control of walking must include consideration of central coupling pathways, such as those proposed for cockroaches by Pearson and Iles (1973), in addition to other coupling mechanisms that may exist. In view of the fact that even the existence of central timing pathways in stick insects has been questioned (Bässler and Wegner, 1983), a broader consequence of this conclusion is that one must be quite careful not to overgeneralise results from one insect to another. It may be that cockroaches and

stick insects are sufficiently different so that no single model will adequately account for walking control in both.

Another important generalisation is that sensory feedback from the legs is largely responsible for the timing of ipsilateral leg pairs during slow walking. This has been amply demonstrated in stick insects (see reviews by Bässler, 1983, 1987; Cruse, 1990), and appropriate pathways have been shown to exist in locusts as well (Macmillan and Kien, 1983). The present papers allow cockroaches to be added to the list.

Coordinating pathways across each ganglion have been demonstrated. In stick insects, these pathways involve mechanisms different from those for ipsilateral coordination (Cruse and Knauth, 1989). The present experiments support this view for the cockroach as well. The detailed mechanisms are not known, but it is clear that amputation has quite a different effect on contralateral coordination from that on ipsilateral coordination. A difference in coordination between contralateral, as opposed to ipsilateral, leg pairs is therefore another organisational principle that may have widespread applicability.

It is worth making one final point about the understanding of walking or of any other behaviour. There are now so many data available on this system that it has become increasingly difficult to integrate and interpret them all. We may soon reach the stage at which only formal simulations of our ideas in the form of operating models will be able adequately to test them.

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