MOTOR ACTIVITY IN THE STUMP OF AN AMPUTATED LEG DURING FREE WALKING IN COCKROACHES

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Summary

1. A rhythmic pattern of motor activity was recorded in the stump of an amputated leg during free walking in cockroaches.

2. During relatively rapid walking, extensor (depressor) and flexor (elevator) muscles in the intact coxa of the amputated leg showed one burst of activity during each cycle of bursting in an adjacent, intact leg. However, during slower walking these muscles could show two or three bursts of activity during each cycle of bursting in an intact leg.

3. Motor bursts in the stump of an amputated leg showed features similar to those of bursts recorded from intact legs. Burst duration increased with an increase in period, and the bursts generally showed consistent timing (phase) relative to bursts in most of the intact legs.

4. The motor pattern recorded in a stump was very like that recorded in an intact leg during walking, and unlike that recorded during searching for a foothold (defined in the text). It is concluded that after the amputation of most of a leg, motor neurones innervating muscles in the stump of the amputated leg continue to be driven by the interneurones that normally drive the intact leg during walking. Analysis of the motor pattern in the stump may therefore reveal important features of the locomotor control system.

Introduction

Leg amputation is an important tool in the study of the coordination of movements in insects. It has been known for more than 150 years that an insect uses a different gait after the removal of most of its two middle legs from that which it uses before the operation (for a review see Delcomyn, 1985c). At present, this altered sequence and timing of leg movements is assumed to be the result of the loss, from the stumps of the legs, of the sensory inputs that signal ground contact and load, since the stumps are too short to touch the walking surface. This assumption is supported by the observation that attaching small wooden sticks to the stumps to replace the missing parts of the legs results in restoration of the normal gait (for a review see Delcomyn, 1985b).

After leg amputation, each stump, in most cases consisting of the coxa and trochanter, continues to move as the insect walks. The coxa moves forwards and backwards in its normal pattern, and the trochanter also continues to flex and extend. The presence in the coxa of a rhythmic motor activity that drives this trochanteral movement has been reported previously (Pearson & Iles, 1973; Delcomyn, 1985a), but the properties of this movement have not been investigated.

Study of the characteristics of the rhythmic activity in the coxal muscles of a leg stump could lead to important insights into the organization of the locomotor control system, including both its central and peripheral (sensory) components. Since the stump lacks all sensory receptors distal to the trochanter, and many of those that still remain are not stimulated with every step as they ordinarily would be (because the stump cannot touch the walking surface), the pattern of rhythmic motor activity in the stump may provide a view of the effects of nearly complete deafferentation of the leg on the central components of the locomotor system.

A crucial question that remains unanswered, however, is whether the rhythmic bursts in the stump represent an attempt by the animal to walk with the stump, or an attempt to find a foothold for a leg that persistently lacks one. Pearson & Franklin (1984) have reported that when a locust walking on rough terrain fails to find a foothold for a leg, the leg engages in searching movements. That is, the leg is raised and lowered and the tibia is extended and flexed in rhythmic movements that are usually effective in locating a solid support for the leg. The rhythmic movements of a leg during searching are similar to the rhythmic movements during walking, but there are differences between the motor patterns that underlie the movements (Delcomyn, 1987). Because of these differences it seems likely that a leg that is searching for a foothold is not functionally connected to the locomotor control system even if the insect continues to walk. Thus, if a cockroach's response to the loss of the leg is to search for a foothold for it, then studying the motor activity in the stump is likely to reveal nothing about locomotion. Since the motor pattern that underlies walking and that which underlies searching can be distinguished (Delcomyn, 1987), examination of the motor pattern in the stump should make it possible to resolve this important issue.

The purpose of the present paper is to describe the basic properties of the motor patterns that can be recorded in the stumps of free-walking American cockroaches. Detailed description of the timing of the bursts in the pattern relative to bursts in other legs will follow in a subsequent report. Motor activity in a leg stump shows many of the same properties as the activity recorded from intact legs. Analysis of these properties further shows that the insect is attempting to use the stump in walking. A preliminary description of some of the results has appeared previously (Delcomyn, 1985a).

Materials and methods

Forty-one male American cockroaches, *Periplaneta americana*, taken from a

colony kept in the Department of Entomology, University of Illinois, were used in these experiments. Animals were prepared for experiments in a standard way (Delcomyn & Usherwood, 1973). Briefly, fine wires were implanted into depressor or elevator trochanteris muscles of the coxa of one or more legs, under CO_2 anaesthesia. These muscles are responsible for depressing (extending) and elevating (flexing) the trochanter, and hence the femur to which the trochanter is fused. Because the middle and rear legs of cockroaches are rotated under the body to a great extent, the depressor and elevator trochanteris muscles are often referred to as extensor and flexor muscles, respectively, so as to reflect more accurately their anatomical function. Most electrode implants were done in extensor muscles of the middle or rear legs, but a few experiments were also carried out on front legs or on flexor muscles.

After electrodes had been implanted, animals were placed in an arena measuring about $25\,cm \times 35\,cm$. The arena was lined with blotting paper to provide good traction, and surrounded with Lucite walls smeared with petroleum jelly to prevent the escape of the animal. Within 5–10 min, the cockroaches seemed well recovered from the anaesthesia, often spontaneously walking about the arena. After a few samples of muscle activity had been recorded during walking by the intact insect, the animal was taken out of the arena and one leg amputated just distal to the junction between the trochanter and the femur. This operation removed the tarsus, the tibia, and nearly all of the femur, leaving the coxa and trochanter intact. Nearly all cockroaches elevated their bodies high enough off the walking surface when they walked so that after amputation the stump of the femur was too short to touch this surface. Walking records from the rare animal in which the stump did touch the walking surface were not analysed. After amputation of a leg the animal was immediately replaced in the arena, and more recordings were taken of motor activity.

Standard recording, display and filming techniques were used. The starts and ends of the bursts of muscle potentials were digitized from filmed records of muscle activity with the aid of a digitizing device (DIGIBIT, Numonics), and the digitized data stored on floppy diskettes using an Osborne I computer.

The data were analysed using custom-written programs and a Zenith 241 computer, as described previously (Delcomyn, 1987). Calculations of phase (a measure of timing between two cyclic events) were made using the ends of the bursts of extensor muscle activity as the reference points. That is, one complete cycle (period) of activity was defined as the time from the end of one burst of extensor activity to the end of the following burst in the same leg. (Burst ends were used because in poor recordings the exact times of their occurrences were somewhat easier to determine than were those of burst starts, and because the step cycle is usually considered to begin with the swing phase, which starts with the termination of an extensor burst.) For computations of phase, the lag between two bursts in different legs was defined as the time from the end of a burst in the leg of interest to the end of the period in the reference leg during which the burst occurred. Phase is defined as the ratio of lag to period.

Results

During brisk to fast free walking over a smooth, level surface, the pattern of motor activity exhibited by muscles in the stump of an amputated leg was quite similar to that seen in an intact leg under similar circumstances (Fig. 1A). That is, extensor and flexor muscles in the stump were active in reciprocal bursts, and during nearly all steps extensor bursts in the stump alternated with extensor bursts in the adjacent legs. Both these features are also present in the motor patterns of intact cockroaches (Delcomyn & Usherwood, 1973; Pearson, 1972). During slower walking, however, some insects exhibited a pattern of multiple bursting in the stump relative to activity in intact legs (Fig. 1B). Visual inspection of the stump revealed that it was moving in time with this activity, swinging through two or three complete cycles of back and forth movement for every single forward and backward movement of the intact legs. The motor pattern in the stump of these slowly moving insects seemed normal in all respects except for this higher frequency of bursts. Extensor and flexor muscles in the stump were still active in bursts that reciprocated with one another, and the overall appearance of the bursts did not allow them to be distinguished from the bursts of intact legs in amputee or intact insects walking under the same conditions.

Twenty-nine of the 41 insects studied (70 %) showed multiple bursting during
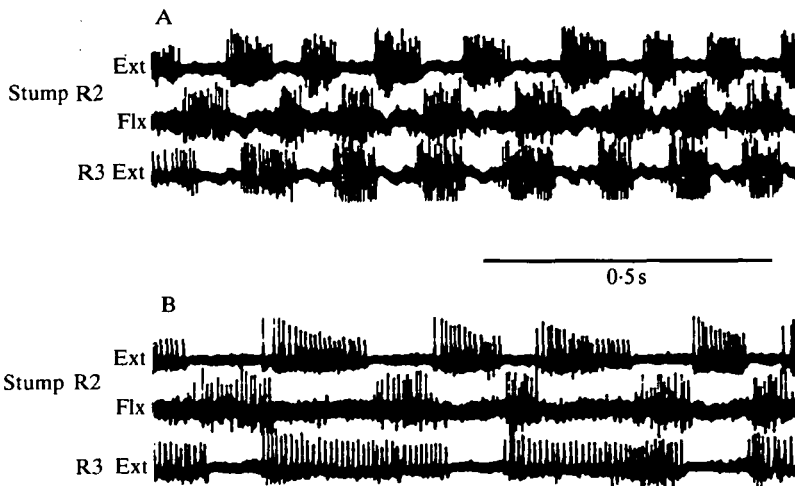

Fig. 1. Records of activity in the coxal muscles that extend (top traces) and flex (middle traces) the femur (extensor and flexor trochanteris muscles) in the stump of an amputated middle leg and of the extensor trochanteris muscle in an intact rear leg on the same side of the body (bottom traces) of a cockroach during free walking. (A) Activity during brisk walking (about 7 steps s^{-1}). Note the alternation of bursts in extensor muscles in the middle and rear legs in most of the steps. (B) Activity during slower walking (about 3 steps s^{-1}). Note that the reciprocal bursts of activity in the flexor and extensor muscles in the coxa of the stump of the amputated middle leg appear at about twice the frequency of the bursts in the intact rear leg. The two records are from the same insect.
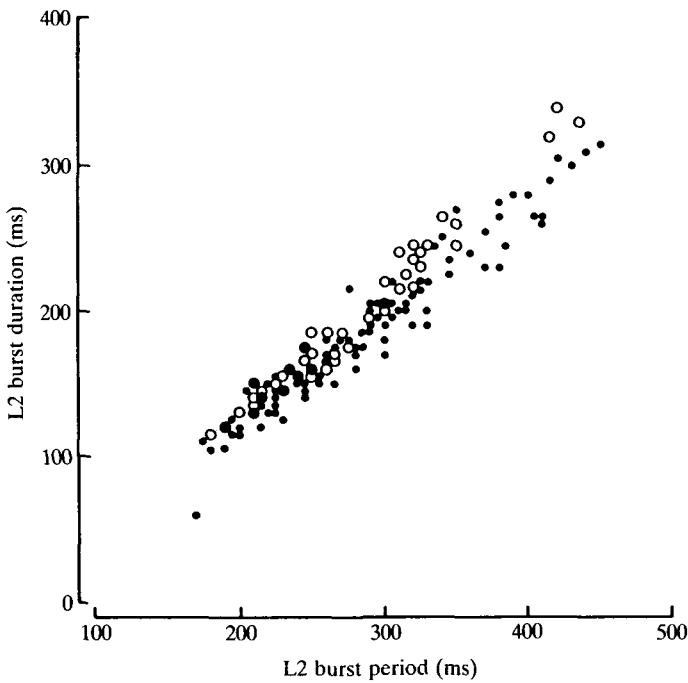
Fig. 2. Scatter plot showing the relationship between burst duration and the duration of the period (measured from burst end to burst end) in extensor trochanteris motor activity in a leg before (open circles) and after (closed circles) most of the leg was amputated at the trochanter–femur joint. A slight decrease in the burst duration corresponding to a given period can be seen after amputation during longer periods.

some steps. All those insects that showed multiple bursting did so during slow walking, but the fastest walking speed at which multiple bursting was present was not the same for all insects. One insect showed multiple bursting at a stepping frequency as high as 11 steps s^{-1} (step period of 90 ms). However, only two insects showed multiple bursting while walking at rates faster than about 5 steps s^{-1} (190–210 ms periods), whereas seven animals showed it at speeds of 4–5 steps s^{-1}. Five steps per second therefore seems to represent the limit above which multiple bursting does not ordinarily occur. Single bursts often continued to occur during some steps while the insect walked slowly. There was no difference between middle leg and rear leg stumps with regard to the appearance of multiple bursting.

The extensor motor bursts recorded from the stumps of amputated legs exhibited features similar to those of bursts in intact legs (Pearson, 1972; Delcomyn & Usherwood, 1973; Delcomyn, 1985a). They were coincident with the stance phase of walking, had clear starting and ending points and exhibited a relatively high frequency of spikes (Fig. 1). Furthermore, they varied in duration as the period of the burst cycle varied (Fig. 2), longer bursts being associated with longer burst cycles. These are also features of bursts in intact legs. The main

difference was that the motor bursts in the stump were somewhat shorter for any given cycle period than normal.

Flexor bursts were examined in the stumps of legs in a few animals. These also showed features similar to those of flexor bursts in intact insects. Flexor bursts are coincident with the interval between extensor bursts and with the swing phase of the step cycle. Just as in intact cockroach legs (Pearson, 1972; Delcomyn & Usherwood, 1973) and in many other insects (stick insects: Graham, 1972; locusts and grasshoppers: Burns, 1973), flexor bursts in the stumps increased in duration as the period of the burst cycle increased (not shown), although with somewhat more scatter than in an intact leg.

The timing of the bursts in the stump was relatively normal as well. In intact cockroaches, motor bursts in adjacent legs tend to maintain almost constant timing (phase) relative to one another (Delcomyn & Usherwood, 1973), just as do movements of the legs (Delcomyn, 1971). Motor bursts in amputated stumps also showed this feature, although with a few differences. During walking in which a stump exhibited only one burst during the cycle of stepping of an adjacent leg, the phase of the stump motor bursts was fairly normal relative to adjacent legs. That is, during brisk or fast walking in all insects and during slow walking in those insects that did not show multiple bursting, bursts in the stump occurred at about the same time relative to an adjacent intact leg as they did before the femur and tibia had been amputated (Fig. 3A,B).

When multiple bursting occurred, timing was not so simple. Computation of the phase between the motor activity in a stump and that in the *contralateral* leg revealed little evidence of any consistent or preferred phase relationship (Fig. 3C), as reported previously (Delcomyn, 1985a). However, the phase between motor activity in a stump and an adjacent *ipsilateral* leg showed two or more strong preferred timing relationships (Fig. 3D); one preferred phase for each of the multiple bursts. In this case the phase was independent of the period of stepping of the intact legs over the entire range of walking speeds during which multiple bursting was exhibited (Fig. 4), just as it is during walking in intact cockroaches (Delcomyn, 1971; Delcomyn & Usherwood, 1973).

The features of the motor pattern in the stump suggested that the motor neurones driving the stump muscles were still coupled to the locomotor control system and being driven by it. Nevertheless, it might have been that, with most of the leg missing, the insects were attempting to search for a foothold that they could never find. Most of the evidence presented above tended to support the view that the stump motor pattern represented walking, not searching. This view was further supported by the observation that, while an insect was walking, the rather slow, continuously modulated pattern that is characteristic of searching movements (Delcomyn, 1987) was absent in both the stump and the intact leg. However, sometimes the motor pattern associated with searching is a bursting rather than a continuously modulated one (Delcomyn, 1987), so that the absence of the modulated motor pattern usually associated with searching is not conclusive. Furthermore, multiple bursting in a leg muscle is not a common feature of

intact walking. Therefore, in spite of the similarities between the motor patterns that can be recorded in a stump and those seen in intact legs, it was still possible that the bursts in the stump represented an attempt by the insect to search for a foothold for the stump, and not an attempt to use it in walking.

To resolve this issue, the bursting motor pattern of a stump was analysed and compared with the motor pattern recorded from an intact leg. It has already been shown that during searching (in intact legs) the bursting pattern that sometimes appears in extensor muscles shows an independence between burst interval and burst period, whereas during walking burst interval increases as burst period increases (Delcomyn, 1987). Plotting the interburst interval (the time from the end of one burst to the start of the next) against the duration of the entire burst cycle (from the end of one burst to the end of the next) for extensor muscle motor patterns recorded from stumps of amputated legs during walking clearly showed that the pattern was very much more like walking than searching. This statement is true for both middle (Fig. 5A) and rear (Fig. 5B) legs. (Front leg amputees were not examined because insects with a front leg amputated were generally able, because of the anatomy and articulation of the front leg, to place the stump on the
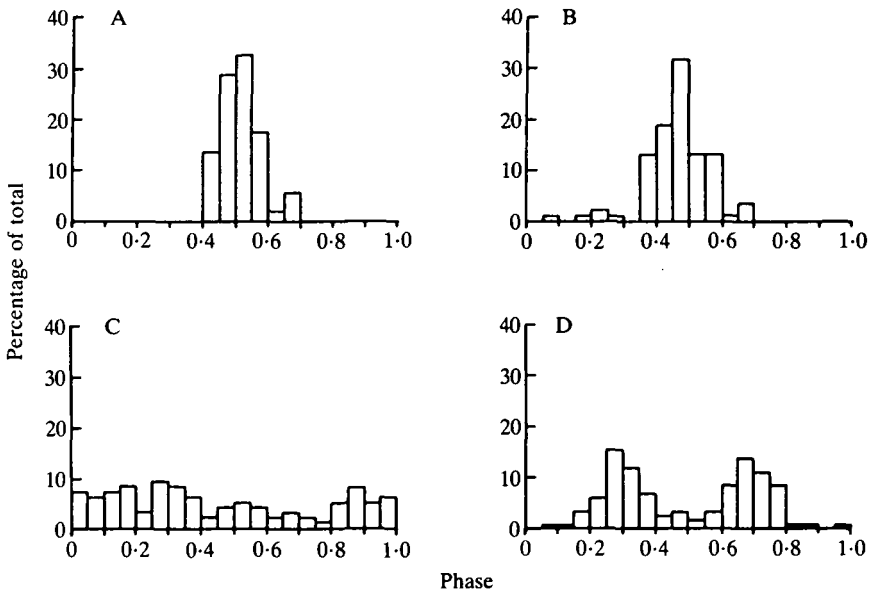

Fig. 3. Normalized frequency histograms showing the distribution of phases between motor bursts in amputee and in intact legs. (A) A typical distribution of phases between a pair of adjacent legs in an intact insect walking freely. (B) Phase between a middle leg stump and the ipsilateral rear leg in an animal that exhibited only a few double bursts. Note the similarity to A. (C) Phase between a middle leg stump and its intact contralateral partner. In this case there is little indication of any preferred phase between the legs. (D) Phase between a middle leg stump and the rear leg on the same side. The peaks represent the preferred timing of the two bursts that occurred in the stump for each step in the intact leg.
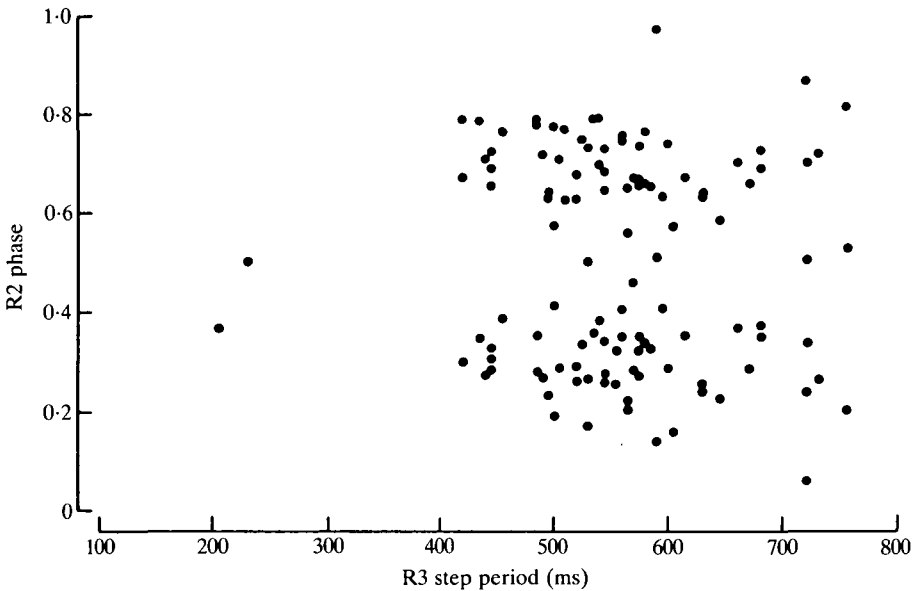
Fig. 4. A scatter plot of the phase of a middle leg stump relative to a rear leg *vs* step period, during multiple bursting. Same animal and legs as in Fig. 3D. Note that phase is independent of step period. Leg R2 was amputated.

walking surface with each step.) Furthermore, scatter plots of the interval *vs* duration relationship in leg stumps showed absolutely no difference between data derived from bursts that were timed one-for-one with those of an intact leg and those that showed double or triple bursts, hence providing further support for the view that multiple bursts were not an expression of a nonlocomotor behaviour.

Discussion

The persistence of rhythmic motor activity in the stump of an amputated leg in spite of the elimination of nearly all the sensory input normally associated with leg movement is not surprising. Rhythmic motor activity has previously been shown to persist not only after deafferentation of some or all legs in cockroaches and stick insects (Pearson & Iles, 1970, 1973; Pearson & Fourtner, 1975; Bässler & Wegner, 1983) but also after total deafferentation of the nervous system in many different animals (Delcomyn, 1980). The motor activity in a stump resembles that in an intact leg. Although this similarity suggests that the insect is still attempting to use the stump to walk, such a conclusion cannot be drawn without further analysis, since there are other rhythmic movements that cockroaches make with their legs, such as searching, righting and grooming (Pearson, 1985).

The activity in the stump does not represent righting because it shows a fairly constant timing relative to activity in intact legs, a feature not seen in righting (Zill, 1986). It does not represent grooming because grooming is an activity that occurs

only at rather low frequencies and when the insect is standing still (Reingold & Camhi, 1977). Besides walking, the most likely behaviour that the motor pattern in the stump could represent is searching. However, as shown in this paper, the pattern shows neither the slow, modulated motor pattern nor the independence between burst interval and burst period that is characteristic of searching (Delcomyn, 1987).

Because multiple bursts, and the multiple steps that the burst pattern generates, are not usual features of insect walking, their occurrence may be thought to present a difficulty for accepting the suggestion that this pattern represents walking. Multiple steps or multiple motor bursts are generally associated with an unusual sensory input, often experimentally induced. They have been noted in stick insects (Foth & Bässler, 1985) and even in lobsters (Chasserat & Clarac, 1980) walking with one or more legs on a motor-driven belt when the belt speed differs from the animal's preferred speed. They have also been reported in stick insects walking on independently loadable treadwheels (Foth & Graham, 1983), on a slippery surface (Epstein & Graham, 1983) or with reversed sensory input in a leg
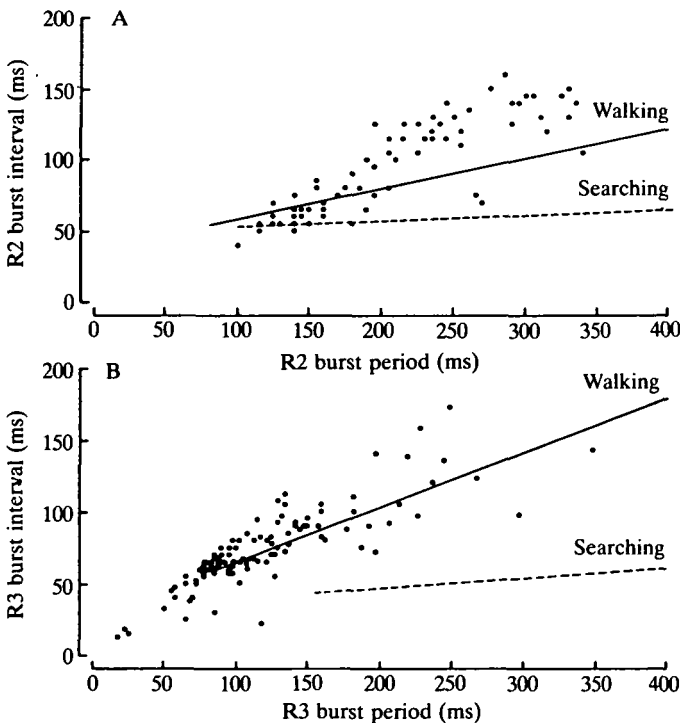

Fig. 5. Scatter plots of the relationship between interburst interval and burst period in the activity of extensor trochanteris muscles in the stumps of amputated middle (A) and rear (B) right legs during walking. The lines in the figures represent the regression lines of the relationship between the same parameters in the same legs during free walking (solid line) and during searching (dashed line) before part of the leg was amputated.

(Graham & Bässler, 1981). Cockroaches walking with deafferented legs, a situation functionally similar to amputation, show multiple bursts in the operated legs (Pearson & Iles, 1973). However, even in intact insects, multiple steps of one or more legs relative to others can be seen at the start or end of walking (Delcomyn, 1981), or in the middle of sequences in katydids, which have unusually long rear legs that typically step only once for every two steps of the front and middle legs (Graham, 1978). In most of these cases, the unusual motor pattern was obviously produced during walking of the affected leg(s). Therefore, in view of the other evidence presented in this paper, the observation of multiple bursting should not preclude the conclusion that the motor pattern in a stump represents an attempt by the insect to walk with the stump.

Given this conclusion, the pattern of activity in the motor neurones innervating the stump is then in part a reflection of the excitation they receive from the interneurones that constitute the central component of the locomotor control system, just as is the pattern of activity in motor neurones innervating intact legs. The main difference is the absence of the influence of sense organs in the missing parts of the leg and, presumably, of the trochanteral mechanoreceptors that no longer receive stimulation *via* ground contact by the leg. Analysis of the motor pattern in a stump alone and in relation to the motor patterns in intact legs may therefore reveal important information about the locomotor control system. For example, the observation that bursts can occur in the stump at a frequency two or three times higher than in an adjacent intact leg suggests that sensory feedback must be an important factor in keeping all the legs stepping at the same rate. This conclusion is supported by all the work cited above showing that an unusual sensory input can induce stepping rates that are different in different legs. However, the maintenance of consistent timing between the bursts in the stump and those in an ipsilateral adjacent leg suggests that there are still strong coupling mechanisms at work, either peripheral, *via* the intact leg, or central. The uncoupling of phase between contralateral leg pairs when multiple bursting occurs may mean that the coupling mechanisms are organized primarily along the length of the body rather than across it.

An interesting feature of the motor patterns recorded in the stumps of amputated legs is that they are not as consistent among individuals as one might expect. That is, some insects show no multiple bursting after amputation, even at quite low speeds of walking. Among insects that do exhibit multiple bursting, some show it only during fairly slow walking, whereas others do so during both slow and more rapid walking. This is a little surprising if one considers the central nervous system of an insect to be 'hard-wired' so that a particular stimulus (or lack of stimulus) should always lead to the same qualitative reaction by the insect. The rather large variability seen in the motor pattern of a stump may be a reflection of important individual differences among cockroaches that have been shown to exist in the locomotor control system (Delcomyn & Cocatre-Zilgien, 1988). It would be interesting to determine whether the differences in the walking speed at which multiple bursting first occurs are correlated with some of the small, but highly

statistically significant, differences in mean phase that have been observed in intact insects.

References

BÄSSLER, U. & WEGNER, U. (1983). Motor output of the denervated thoracic ventral nerve cord in the stick insect *Carausius morosus*. *J. exp. Biol.* **105**, 127–145.

BURNS, M. D. (1973). The control of walking in Orthoptera. I. Leg movements in normal walking. *J. exp. Biol.* **58**, 45–58.

CHASSERAT, C. & CLARAC, F. (1980). Interlimb coordinating factors during driven walking in Crustacea. A comparative study of absolute and relative coordination. *J. comp. Physiol.* **139**, 293–306.

DELCOMYN, F. (1971). The locomotion of the cockroach *Periplaneta americana*. *J. exp. Biol.* **54**, 433–452.

DELCOMYN, F. (1980). Neural basis of rhythmic behavior in animals. *Science* **210**, 492–498.

DELCOMYN, F. (1981). Insect locomotion on land. In *Locomotion and Exercise in Arthropods* (ed. C. F. Herreid & C. R. Fourtner), pp. 103–126. New York: Plenum Press.

DELCOMYN, F. (1985a). Sense organs and the pattern of motor activity during walking in the American cockroach. In *Insect Locomotion* (ed. M. Gewecke & G. Wendler), pp. 87–96. Hamburg: Paul Parey.

DELCOMYN, F. (1985b). Factors regulating insect walking. *A. Rev. Entomol.* **30**, 239–256.

DELCOMYN, F. (1985c). Walking and running. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 5 (ed. G. A. Kerkut & L. I. Gilbert), pp. 439–466. London: Pergamon Press.

DELCOMYN, F. (1987). Motor activity during searching and walking movements of cockroach legs. *J. exp. Biol.* **133**, 111–120.

DELCOMYN, F. & COCATRE-ZILGIEN, J. H. (1988). Individual differences and variability in the timing of motor activity during walking in insects. *Biol. Cybernetics* (in press).

DELCOMYN, F. & USHERWOOD, P. N. R. (1973). Motor activity during walking in the cockroach *Periplaneta americana*. I. Free walking. *J. exp. Biol.* **59**, 629–642.

EPSTEIN, S. & GRAHAM, D. (1983). Behaviour and motor output of stick insects walking on a slippery surface. I. Forward walking. *J. exp. Biol.* **105**, 215–229.

FOTH, E. & BÄSSLER, U. (1985). Leg movements of stick insects walking with five legs on a treadwheel and with one leg on a motor-driven belt. II. Leg coordination when step-frequencies differ from leg to leg. *Biol. Cybernetics* **51**, 319–324.

FOTH, E. & GRAHAM, D. (1983). Influence of loading parallel to the body axis on the walking coordination of an insect. II. Contralateral effects. *Biol. Cybernetics* **48**, 149–157.

GRAHAM, D. (1972). A behavioural analysis of the temporal organisation of walking movements in the 1st instar and adult stick insect (*Carausius morosus*). *J. comp. Physiol.* **81**, 23–52.

GRAHAM, D. (1978). Unusual step patterns in the free walking grasshopper *Neoconocephalus robustus*. I. General features of the step patterns. *J. exp. Biol.* **73**, 147–157.

GRAHAM, D. & BÄSSLER, U. (1981). Effects of afference sign reversal on motor activity in walking stick insects (*Carausius morosus*). *J. exp. Biol.* **91**, 179–193.

PEARSON, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173–193.

PEARSON, K. G. (1985). Are there central pattern generators for walking and flight in insects? In *Feedback and Motor Control in Invertebrates and Vertebrates* (ed. W. J. P. Barnes & M. H. Gladden), pp. 307–315. London: Croom Helm.

Pearson, K. G. & Fourtner, C. R. (1975). Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol.* **38**, 33–52.

Pearson, K. G. & Franklin, R. (1984). Characteristics of leg movement and patterns of coordination in locusts walking on rough terrain. *Int. J. Robotics Res.* **3**, 101–112.

Pearson, K. G. & Iles, J. F. (1970). Discharge patterns of coxal levator and depressor motorneurones of the cockroach, *Periplaneta americana. J. exp. Biol.* **52**, 139–165.

Pearson, K. G. & Iles, J. F. (1973). Nervous mechanisms underlying intersegmental co-ordination of leg movements during walking in the cockroach. *J. exp. Biol.* **58**, 725–744.

Reingold, S. C. & Camhi, J. M. (1977). A quantitative analysis of rhythmic leg movements during three different behaviors in the cockroach, *Periplaneta americana. J. Insect Physiol.* **23**, 1407–1420.

Zill, S. N. (1986). A model of pattern generation of cockroach walking reconsidered. *J. Neurobiol.* **17**, 317–328.