J. exp. Biol. **133**, 111–120 (1987)
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MOTOR ACTIVITY DURING SEARCHING AND WALKING MOVEMENTS OF COCKROACH LEGS

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Accepted 22 May 1987

SUMMARY

1. Rhythmic motor activity may be recorded in the legs of cockroaches during the execution of several different types of behaviour that involve leg movements. It was examined in detail during searching and walking.

2. During walking, motor activity always consisted of a series of bursts separated by silent periods. During searching, it was usually continual, but modulated in frequency.

3. Sometimes, the motor pattern recorded from a searching leg was burst-like rather than modulated. In these cases, it could nevertheless be reliably distinguished from the motor pattern recorded during walking by a simple analysis of the burst pattern.

4. An analysis of the motor pattern recorded during righting indicated that this pattern was more like that for walking than that for searching. Therefore, searching is not simply walking that lacks certain periodic sensory input due to leg contact with the ground.

5. It is concluded that walking and searching can be reliably distinguished from one another on the basis of an analysis of a record of motor activity in a single leg muscle only. An ability to distinguish between similar types of behaviour on the basis of the motor pattern may prove useful in a variety of experiments.

INTRODUCTION

Studies of the physiological basis of rhythmic behaviour, such as feeding and walking, are frequently carried out on restrained and dissected animals. In such studies, the presence of a rhythmic motor pattern in appropriate muscles or motor nerves is generally taken as an indication that the animal is engaged in the behaviour under study. In recent years, however, there have been challenges to the tacit assumption that rhythmic activity in a particular set of muscles is an indicator of only one specific type of behaviour. These challenges have been based on the recognition that there may be more than one behaviour that involves rhythmic activity in a given group of muscles, and that superficially similar motor patterns may underlie behaviour patterns that are different from each other (Pearson, 1985; McClellan, 1982*a*,*b*).

Key words: *Periplaneta americana*, cockroach, motor pattern, rhythmic behaviour, leg movement.

Insect walking is a repetitive behaviour that has been investigated physiologically (e.g. Pearson & Fourtner, 1975; Fourtner, 1976). In most of these investigations, however, little attempt was made to demonstrate quantitatively that the motor patterns used to monitor locomotor activity actually represented walking, and recent reports have questioned the validity of considering the patterns obtained from pinned-out insects to represent walking (Zill, 1986). Since insects may move their legs rhythmically during grooming, righting and searching as well as during walking, it is clear that a rhythmic motor pattern in leg muscles could represent any one of these activities.

Since studies of motor patterns recorded from the stumps of amputated legs in cockroaches during free walking have shown some unusual properties (Delcomyn, 1985), it seems important to be able to distinguish the motor pattern associated with walking from the patterns associated with other rhythmic movements of the legs. Motor patterns associated with grooming and righting have already been studied (Reingold & Camhi, 1977; Sherman, Novotny & Camhi, 1977; Zill, 1986), but those associated with searching have not. I therefore compared the patterns of rhythmic motor activity in the muscles of legs of freely walking cockroaches with those obtained when a leg was searching for a foothold. The patterns were usually different, but when they were not they could be distinguished on the basis of a quantitative parameter of the motor activity.

MATERIALS AND METHODS

Male cockroaches, *Periplaneta americana*, from a colony at the University of Illinois were used. Electromyographic (EMG) recordings were made of activity in coxal muscles of front, middle or rear legs, using standard methods (Delcomyn & Usherwood, 1973). Most recordings were taken from the muscle that extends the trochanter (and therefore the femur, to which the trochanter is fused), but a few recordings were made from flexor muscles as well.

Recordings were taken from 43 insects during free walking and during searching. After recording wires had been implanted, an animal was first allowed to walk freely on rough paper in an enclosed arena about 25 cm × 35 cm, while recordings were taken of muscle activity. The insect was then impaled on a pair of large pins, inserted just under the dorsal cuticle. The pins were fastened in a holder, and the insect was positioned over a glass plate. The animal was induced to search by removing the glass plate. Sometimes the plate was arranged so that the animal could reach it with only a few of its legs. Under these circumstances the remaining legs often searched. After an adequate sample of motor activity during searching had been recorded, some animals were removed from the pins, set back in the arena, and allowed to walk freely again. There was no qualitative or quantitative difference between motor patterns recorded from freely walking cockroaches before and after searching had been induced.

Righting was evoked in a few insects by flipping the animal on its back as it stood or walked in the arena. In other animals it occurred spontaneously after the insect had fallen off one of the greased walls enclosing the arena. Motor patterns were recorded during righting in six different animals.

Motor patterns were recorded conventionally and stored on tape for later playback. Some recordings were stored on standard VHS video cassettes using a modified video cassette recorder (model 420 B, Vetter, Rebersburg, PA). Records to be analysed were photographed with a Grass Kymograph camera using Kodak Kind 1930 photographically sensitive paper (Visual Data Systems, Chicago). Filmed records were analysed with the aid of an electronic digitizing device (Numonics, Lansdale, PA). Events of interest were marked with the device, transmitted to an Osborne 1 microcomputer and analysed.

RESULTS

Motor patterns recorded from extensor muscles during free, unrestrained walking typically show well-defined bursts of activity (Fig. 1A). In addition, simultaneous recordings taken from homologous muscles in two adjacent legs show an alternation in the activity of the two muscles. An analysis of the temporal organization of the motor pattern reveals further features, such as an increase in burst duration and interburst interval as the burst period increases (that is, as the animal walks more slowly). These features are already well-known (Pearson, 1972; Delcomyn & Usherwood, 1973).

Most of the motor patterns recorded during searching were quite different. Rather than a clear bursting pattern like that seen during walking, they typically showed a low-frequency activity that was continually modulated (Fig. 1B). However, in a few animals a bursting pattern of motor activity superficially similar to that seen during free walking was observed (Fig. 1C). These bursting patterns were usually characterized by a lower frequency of impulses in each burst and a less clearly defined start and end of each burst than in free walking. Furthermore, simultaneous recordings from homologous muscles in adjacent legs showed a variable temporal relationship between the bursts of activity in the two legs, quite different from the alternating activity seen during free walking (cf. Fig. 1A and 1C).

With some experience it was possible to distinguish the bursting motor patterns recorded from a single leg during searching from those associated with walking. However, it was important to have a simple, quantitative measure by which the two could be distinguished without the necessity for any previous experience. The duration of the interval between the end of one burst and the start of the next, as a function of the cycle (period) of burst activity, was a parameter that differed between walking and searching, and was therefore subjected to detailed analysis. (Since the burst period is the sum of the interburst interval and the burst duration, the latter also differed in the two types of behaviour. However, variations in the intervals are much easier to see because intervals are generally quite short relative to the corresponding period.)

Analysis of bursting patterns obviously required that a good sample of bursting activity associated with searching be available. Suspension of the animal and removal

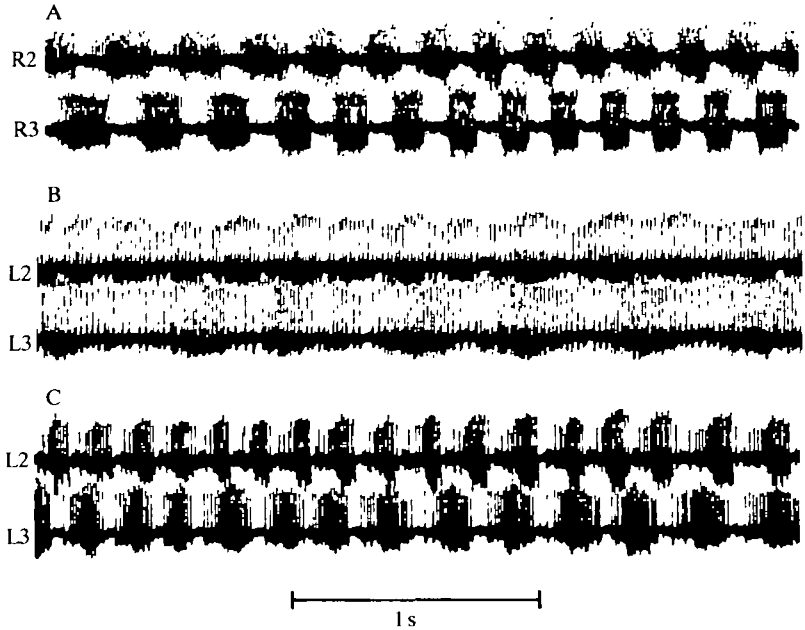

Fig. 1. Records of activity in the coxal muscles that extend the femur (extensor femoralis muscles) in the legs of cockroaches. (A) Activity in the right middle and rear leg extensor femoralis muscles during free walking. Note the high frequency of activity within each burst, the clear definition of bursts and the strict alternation between bursts in the two legs. (B) Activity in left middle and rear leg extensor femoralis muscles during searching, showing the characteristic low-frequency, modulated activity associated with searching. (C) Bursting activity during searching, characterized by a lower frequency of activity in each burst, less clear definition of the burst and variable timing between bursts in adjacent legs, compared to bursting activity during free walking.

of support from all its legs simultaneously almost always induced a modulated rather than a burst-like pattern of activity. However, removing support from only some of the legs induced burst-like activity in some (about half) of the animals. Usually, burst-like activity was associated with more vigorous movements. When an insect was unable to find support after some minutes, its movements generally became less vigorous, and the modulated motor activity more characteristic of searching appeared.

Examination of interburst interval *vs* burst period for front, middle and rear legs showed that the relationship between the parameters in each leg differed between free walking and searching. In front legs (Fig. 2A), interburst intervals tended to be longer for a given burst period during searching than during free walking. In middle (Fig. 2B) and rear legs (Fig. 2C), however, the reverse was true, interburst intervals being shorter for a given burst period during searching than during walking. Furthermore, in middle and rear legs, not only were interburst intervals relatively

Fig. 2. Scatter plots of the relationship between interburst interval and burst period in the activity of extensor femoralis muscles in animals during searching (○) and walking (●) movements of the legs from which the recordings are taken.

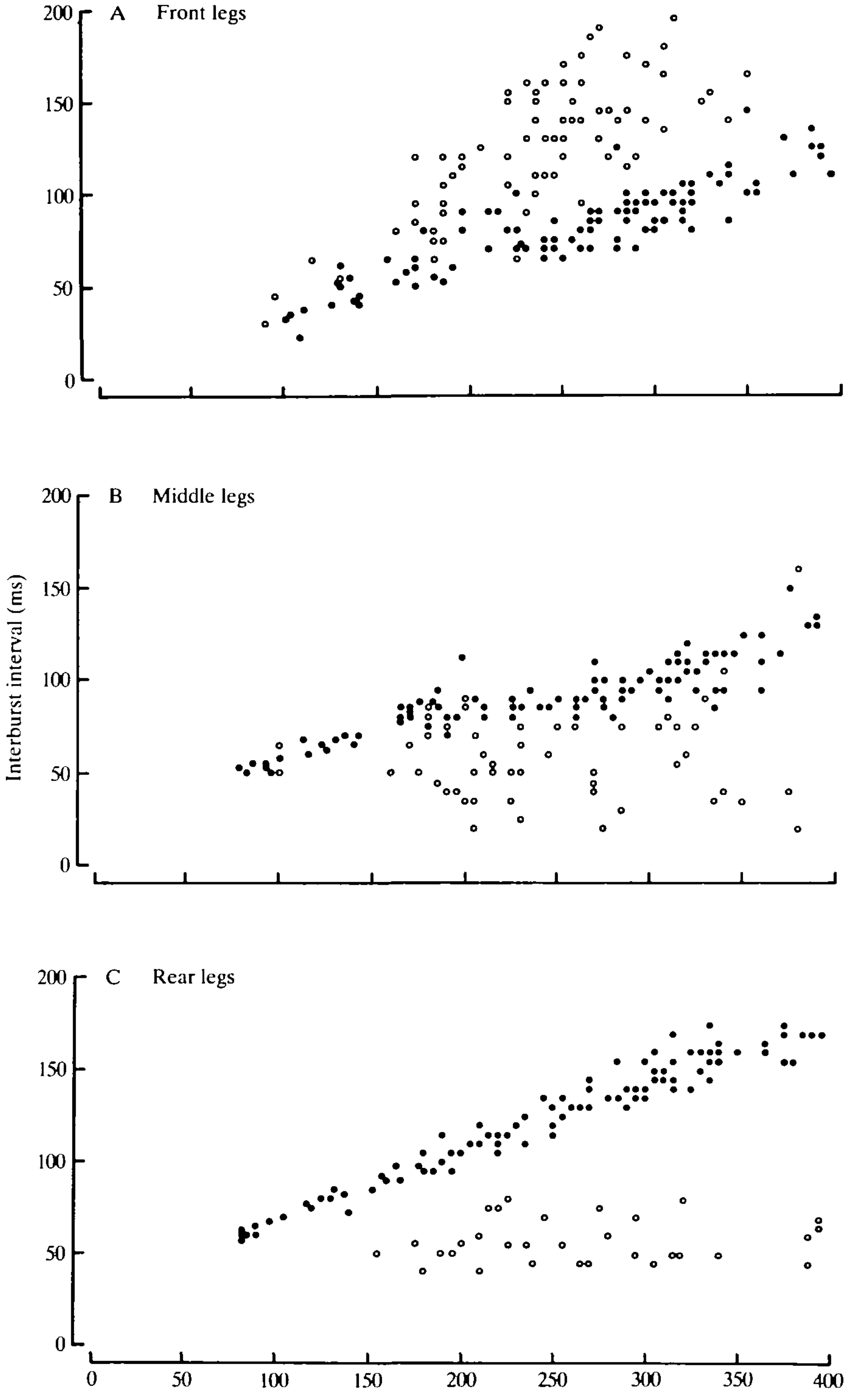
A Front legs
B Middle legs
C Rear legs
Interburst interval (ms)
Burst period (ms)
0
50
100
150
200
250
300
350
400

Fig. 2

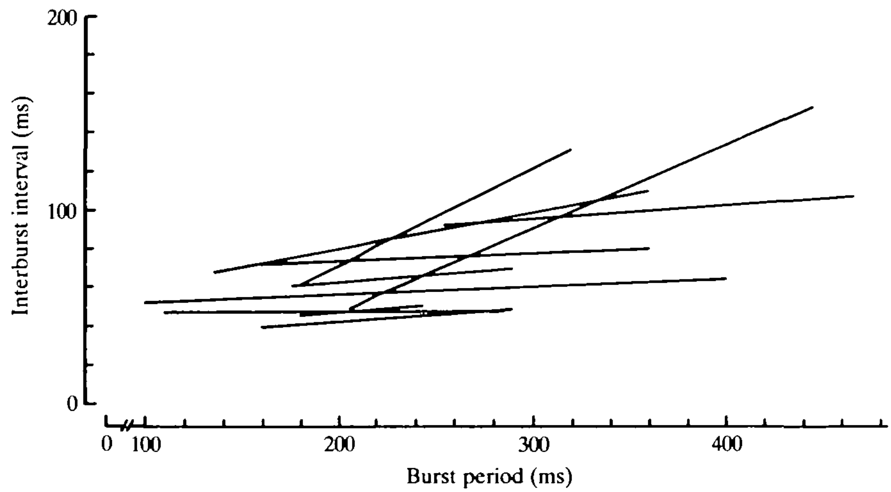

Fig. 3. Calculated regression lines for all the records of bursting activity obtained from middle legs (R2 or L2) during searching movements of those legs. The beginning and end points of the lines represent the minimum and maximum burst periods recorded for the animal from which each set of data was obtained.

short, in most animals they were entirely independent of burst period. There is no obvious reason for the different behaviour of front leg interburst intervals compared to those in middle and rear legs, but it should be noted that the front legs in cockroaches are used somewhat differently from the other two sets of legs. This difference in use may involve a somewhat different neural organization in the prothoracic ganglion which, in turn, may lead to a different motor effect of searching from that seen in the other legs.

Analysis of interburst intervals allows one to distinguish between searching and walking behaviour. The analysis is reliable, but not perfect, since occasional animals are encountered in which interburst interval varies during searching as it does during free walking. For example, Fig. 3 shows the calculated regression lines of interburst interval *vs* burst period for all the motor patterns recorded during searching from a right or left middle leg. Each line represents searching data from a different animal. Only two of the ten animals represented here showed interval *vs* period distributions that were similar to those normally seen during free walking.

Flexor muscle activity during searching was examined in some animals, and generally showed properties similar to that of extensor muscles (not shown). In flexor muscles as well, a continually modulated pattern of activity was most common during searching, with bursting patterns observed in some insects. In flexor muscles the duration of the burst was generally short compared to the duration of the interburst interval, so burst duration rather than interburst interval was plotted as a function of burst period. Comparing flexor burst duration in free-walking and searching legs usually yielded results similar to those described above for interburst intervals in extensor records. That is, there was a tendency for the burst duration to be independent of burst period during searching but not during walking. However, in a substantial minority of cases, there was little difference between the motor patterns recorded during the two types of behaviour, so that they could not reliably be distinguished from each other by analysis of flexor records.

The results described above suggested that walking and searching could each be evoked by appropriate central nervous system action. However, another possibility was that the CNS generated only a single 'behaviour', and that the differences in motor patterns seen during walking and searching were due to differences in sensory feedback from the legs when either behaviour was evoked. To assess the contribution of sensory input to the motor pattern, examination was made of the motor pattern that accompanies righting, another behaviour that involves rhythmic leg movements. Righting can be evoked by flipping an insect onto its back. In consists of rapid, laterally directed flexion–extension movements of most of the legs, movements that usually result in flipping the animal right-side up again when one or more tarsi catch the surface on which the animal is lying. At the start of righting behaviour, the moving legs lack any tarsal contact with the walking surface, and lack input from cuticular stress receptors during the extension phase of their movement cycle, although the femur of a leg may make contact with the substrate during the flexion phase.

Righting behaviour has been studied extensively by Reingold & Camhi (1977), Sherman *et al.* (1977) and Zill (1986), so only a few samples were obtained for the present study. Examination of motor patterns recorded during righting behaviour revealed more walking-like than searching-like features. First, the slow, modulated motor activity so characteristic of searching was rarely seen during righting, coming most often after the insect had been on its back for some time and was not engaged in the extremely vigorous movements most commonly characteristic of attempts to right. Second, analysis of the motor patterns in moving legs during righting yielded walking-like variations in interburst interval as a function of burst period. Fig. 4 shows a scatter plot of interburst intervals as a function of burst periods for a middle leg while one insect was on its back trying to right itself. For comparison, the regression line calculated for the interburst interval *vs* burst period data shown in Fig. 2B (recorded while that same insect was searching with its middle leg) has been included in the figure.

These results are consistent with the quantitative analysis of motor patterns recorded during righting that was carried out by Reingold & Camhi (1977). In their fig. 8 they present a plot of mean burst duration as a function of burst period in extensor femoralis muscles in rear legs during righting, the same muscles whose activity was analysed in the results reported here. By using the data in their graph, one can calculate the mean interburst interval (interburst interval = burst period − burst duration) associated with each burst period. Plotting these intervals against burst periods (Fig. 4, open circles) shows that the motor patterns recorded by Reingold & Camhi during righting are similar to those reported here to occur during righting, and not at all like the motor patterns that occur during searching.

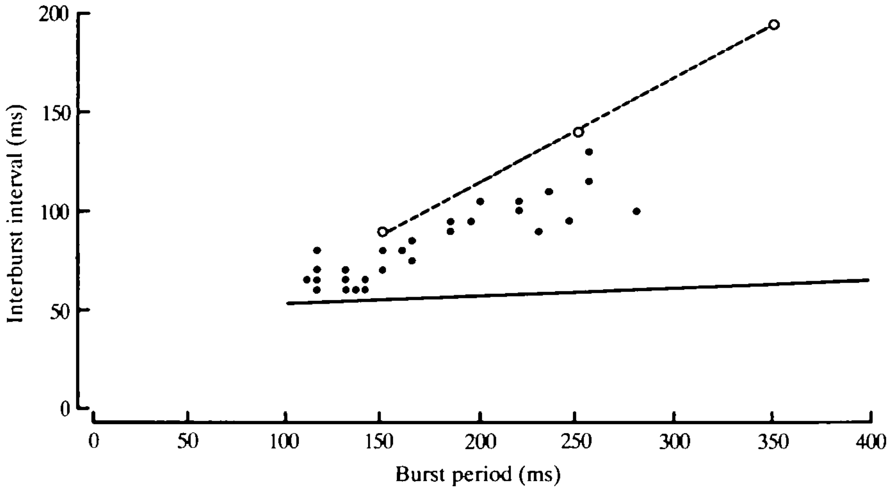

Fig. 4. Scatter plot (solid circles) of the relationship between interburst interval and burst period in a middle leg extensor femoralis muscle in an animal on its back trying to right itself. The solid, nearly horizontal line is the calculated regression line for the data taken during searching (data shown in Fig. 2B). The open circles represent the average interburst interval calculated from data in Reingold & Camhi (1977) (see text). The dashed line was fitted to these circles by eye.

Motor patterns recorded from insect legs during walking showed distinct bursts rather than modulated activity, as found in all published reports except the early one of Hoyle (1964). It is possible that Hoyle's recording methods obscured the underlying bursting motor pattern. In searching legs, the most common motor pattern was a continual, modulated output quite unlike the bursting pattern associated with walking. When a burst-like pattern was observed during searching, it could be distinguished by a simple analysis from that seen during walking.

In recent years there has been some discussion of the nature of the pattern generators for rhythmic types of behaviour. Reingold & Camhi (1977) suggested that walking, searching, righting and grooming were all controlled by a single central pattern generator (CPG), its activity suitably modified by sensory feedback from the moving leg or legs. This has been questioned by Zill (1986). Furthermore, Pearson (1985) has questioned whether walking really can be said to be controlled by any CPG at all. One of the great strengths of the test described here is that the view one takes on this issue cannot influence its experimental application, since it is entirely objective, and based only on the recorded output under specific experimental conditions. The only requirement is that several samples of motor patterns at different rates of leg movement (periods) be taken.

The clear difference shown between searching and walking motor patterns suggests that walking and searching are distinct types of behaviour. That is, the animal may choose to use its legs in a walking mode or a searching mode, as may be appropriate. The different sensory conditions accompanying the two types of behaviour certainly contribute to the differences between the motor patterns, but this difference in sensory feedback does not seem to be the only factor. Righting is a behaviour that shares many features with searching: there is no tarsal contact (at least initially), and therefore no periodic sensory input from cuticular stress receptors that signal load on the leg when the extensor muscles are active. Furthermore, there is little consistent relationship between the phase of motor activity in one leg and that in another during righting (Zill, 1986), just as there is no relationship during searching. Yet, the simple test applied in the experiments reported here suggests that righting is more like walking than it is like searching and that, therefore, searching is quite a different activity from either righting or walking. Subjective impressions support this view. Leg movements during righting are usually quite rapid, as they may be during walking. In contrast, leg movements during searching are generally much more leisurely.

In experiments in reduced preparations, designed to elucidate the cellular mechanisms that underlie the generation of a particular behaviour, it is important to be able to identify the motor patterns responsible for the behaviour. Subtle differences in motor patterns that seem superficially similar may be significant in experiments designed to investigate the neural network underlying the generation of a particular motor pattern. The simple analysis described in this paper may therefore be useful in distinguishing one type of motor pattern from another in animals in which it is not immediately obvious in what behaviour the animal is engaged.

This work was supported in part by Biomedical Research grant BRSG SO7 RR 07030 from NIH, and by NIH grant NS21951. I thank Dennis Andersen and Jan Cocatre-Zilgien for their assistance with some of the experiments.

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