NERVOUS MECHANISMS UNDERLYING INTERSEGMENTAL CO-ORDINATION OF LEG MOVEMENTS DURING WALKING IN THE COCKROACH

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INTRODUCTION

The locomotory leg movements of the cockroach, *Periplaneta americana*, have been fully described in intact animals and in animals following the amputation of various legs (Delcomyn, 1971 *a, b*). For all but the slowest speeds of progression (less than about 5 cm/sec or 3 steps/sec) the intact cockroach uses an alternating tripod gait in which stepping (protraction) of the forelegs and hind legs on one side and the contra-lateral middle leg alternates with the protraction of the remaining three legs. The average phase of protraction of a middle and a fore leg in the cycle of the ipsilateral hind leg is 0·48 and 0·95 respectively. The tripod gait is maintained over a wide range of walking speeds because as the speed of locomotion increases there is a decrease in the protraction time of all legs and in the interval between the end of protraction in one leg and the beginning of protraction in the adjacent ipsilateral leg(s). A close correlation between behavioural and electrophysiological measurements for the move-ments of the hind leg has been reported (Pearson, 1972), but at present there is no electrophysiological data on the relations between the movements of the different legs. The initial part of the current investigation was concerned with describing the dis-charge patterns of motoneurones active during protraction of the hind and middle legs.

The nervous mechanisms responsible for co-ordinating leg movements are very poorly understood. The main experimental approach aimed at obtaining an under-standing of these mechanisms has been to amputate various legs, or parts of legs, and observe the subsequent leg movements (Delcomyn, 1971*b*; Wendler, 1966; Hughes, 1957; Wilson, 1966). The most interesting observation from these experiments is that following removal of the middle pair of legs at the trochanter there is an immediate alteration of the stepping pattern of the remaining four legs. During slow walking in the cockroach a foreleg now steps a short time after the ipsilateral hind leg rather than almost simultaneously. At high rates of running, however, both legs protract almost simultaneously (Delcomyn, 1971*b*; Wilson, 1966). Therefore the phase of the pro-traction of a foreleg in the cycle of the ipsilateral hind leg in these animals depends strongly on walking speed, varying from 0·15 when slowly walking to 1·06 when rapidly running. This immediate change in the pattern of leg movements demonstrates that sensory input from the receptors of the middle leg is important in co-ordinating the movements of the forelegs and hind legs (Delcomyn, 1971*b*). However, the receptors responsible have not been identified and how the lack of sensory input modifies the

sequence of stepping in the remaining legs is not known. Before a satisfactory explana-
tion of the effects of middle-leg amputation can be given it would appear necessary
that we have an understanding of the nervous mechanisms involved in co-ordinating
the leg movements of normal animals.

   The use of electrophysiological techniques has yielded considerable information on
the nervous control of rhythmic movements of single legs (Pearson & Iles, 1970;
Pearson, 1972), but until now these techniques have not been used in study directly
aimed at obtaining information about the mechanisms of co-ordination. The results
of the electrophysiological experiments reported in this paper suggest a plausible
explanation of the nervous mechanisms co-ordinating leg movements during normal
walking and following amputation of the middle legs.

MATERIALS AND METHODS

   All experiments were performed on adult male cockroaches, *Periplaneta americana*.
In both the metathoracic and mesothoracic legs of these animals flexion movements
of the femur occur during the protraction, or stepping, phase due to rapid contractions
in the posterior coxal levator muscles (Pearson, 1972). The innervation of these
muscles and their antagonists, the coxal depressor muscles, has been described
elsewhere (Pearson & Bergman, 1969; Pearson & Iles, 1971). The activity in moto-
neurones supplying the levator muscles was recorded in freely walking animals by
implanting bipolar recording electrodes into the muscles of the metathoracic and
mesothoracic legs. The procedure for implanting the electrodes in the metathoracic
leg has been described earlier (Pearson, 1972). The same procedure was used for the
mesothoracic leg since the gross anatomy and neuromuscular organization is identical
in the two segments (Carbonell, 1947; Pipa & Cook, 1959; Iles, 1972).

   Activity in motoneurones supplying the posterior coxal levator muscles was observed
in restrained dissected preparations by recording directly from the nerve to these
muscles (nerve 6Br4, Pipa & Cook, 1959) with fine silver wire-hook electrodes. To
investigate the possibility of central coupling between the meta- and mesothoracic
ganglia the activity in nerve 6Br4 in both segments was recorded after removal of
all sensory input from leg receptors. The procedure for de-afferenting the thoracic
ganglia, obtaining stable nerve recordings and analysing burst activity have all been
described elsewhere (Pearson & Iles, 1970).

   In order to record from filaments split from the connectives joining the meso- and
metathoracic ganglia, animals were first pinned ventral side up on a narrow cork
board, and the cuticle was removed from above the connective joining the two ganglia.
The sheath surrounding one connective was gently removed using very sharp forceps.
The connective was then cut close to either the mesothoracic or the metathoracic
ganglion and split into about six filaments. Each filament in turn was placed on a fine
silver wire recording electrode and further split if found to contain units whose
activity was correlated with burst activity in motoneurones to the ipsilateral leg
muscles. In this manner small filaments were isolated which contained axons dis-
charging in phase with the flexor bursts in either the mesothoracic or the metathoracic
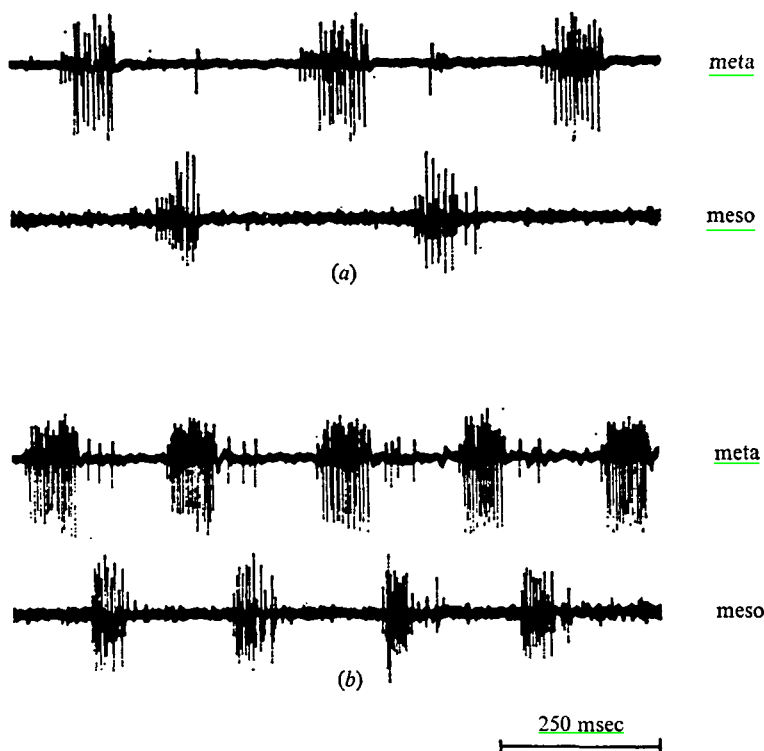ganglion.

Fig. 1. Activity recorded from the coxal levator muscles of the metathoracic leg (top traces) and the ipsilateral mesothoracic leg (bottom traces) during normal walking at two different speeds. Each burst occurs during the protraction phase of leg movement. The small and large junctional potentials in the bursts in both records correspond to activity in motor axons 5 and 6 respectively. The few junctional potentials observed in the metathoracic records during the mesothoracic bursts arise from activity of an unidentified motor unit whose function is unknown. Note that the mesothoracic bursts have a slightly shorter duration than the metathoracic bursts.

RESULTS

(1) *Recordings from walking animals*

A major aim of the current investigation was to determine the nervous mechanisms controlling the sequence of protraction (stepping) in the different legs during normal walking. Thus it was important to determine first the patterns of activity in motoneurones discharging during protraction. In the meso- and metathoracic legs protraction is largely the result of a rapid flexion movement of the femur relative to the coxa. For leg movements less than 10 steps/sec it has been reported that these movements are produced by bursts of activity in two slow excitatory motor axons (axons 5 and 6) supplying the posterior coxal levator muscles (Pearson, 1972). This is also true in the mesothoracic legs. Figure 1 shows bursts of activity recorded simultaneously from the posterior coxal levator muscles in ipsilateral meta- and mesothoracic legs of an animal freely walking at two different speeds. At the two walking speeds shown it is clear that in each leg two motor axons discharge in a short high-frequency burst. The mesothoracic burst durations are shorter than those of the metathoracic, a finding
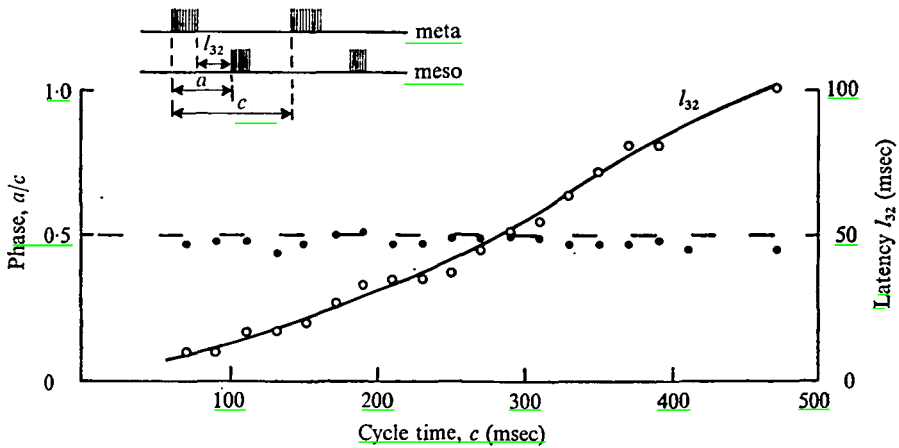
Fig. 2. Phase of the mesothoracic levator bursts in the cycle of the metathoracic levator bursts, a/c (●), and the duration between the end of the metathoracic levator bursts and the beginning of the mesothoracic bursts, b (○), as a function of metathoracic cycle time, c. Cycle time was divided into periods of 20 msec width and each point is the average of at least three measurements within each period. The horizontal dashed line represents the phase value of 0·5.

which corresponds to the behavioural observation that the protraction time of the mesothoracic leg is shorter than that of the metathoracic (Delcomyn, 1971 a). The magnitudes of the burst durations of the smaller unit in each segment also correspond closely to the time of protraction in each leg measured by Delcomyn (1971 a).

Of particular interest in the present study was the relative timing of levator bursts in the two segments. As the walking speed increased there was a decrease in the durations of the levator bursts (Pearson, 1972; figure 1) and a decrease in the interval between the end of a burst in one segment and the beginning of the next burst in the other segment (Fig. 2). These changes result in a fairly constant phase relation between the beginning of the mesothoracic burst and the metathoracic cycle over a wide range of walking speeds. Fig. 2 shows this phase relation in a typical preparation for walking speeds from 2 to 10 steps/sec. Over this range the average phase was slightly less than 0·5 for most of the observed speeds. The mean value for all speeds of walking greater than 2 steps/sec was 0·48. This corresponds very closely to the value measured by Delcomyn (1971a) for the phase of protraction of R_2 in the cycle of R_3.

(2) Recordings from de-afferented preparations

In an attempt to establish that central mechanisms are involved in co-ordinating leg movements the activity of motor axons supplying the posterior coxal levator muscles of ipsilateral meso- and metathoracic legs was recorded after removal of all sensory input from leg receptors. Since a high level of burst activity in levator moto-neurones was desirable when looking for any correlation between activity in the two segments these experiments were performed either on headless animals or on animals after removal of the supraoesophageal ganglion (brainless animals). Brainless cock-roaches readily walk using the normal tripod gait (a similar observation has been reported for mantids; Roeder, 1937) while in headless animals alternate stepping movements of ipsilateral meso- and metathoracic legs often occur for a few cycles and
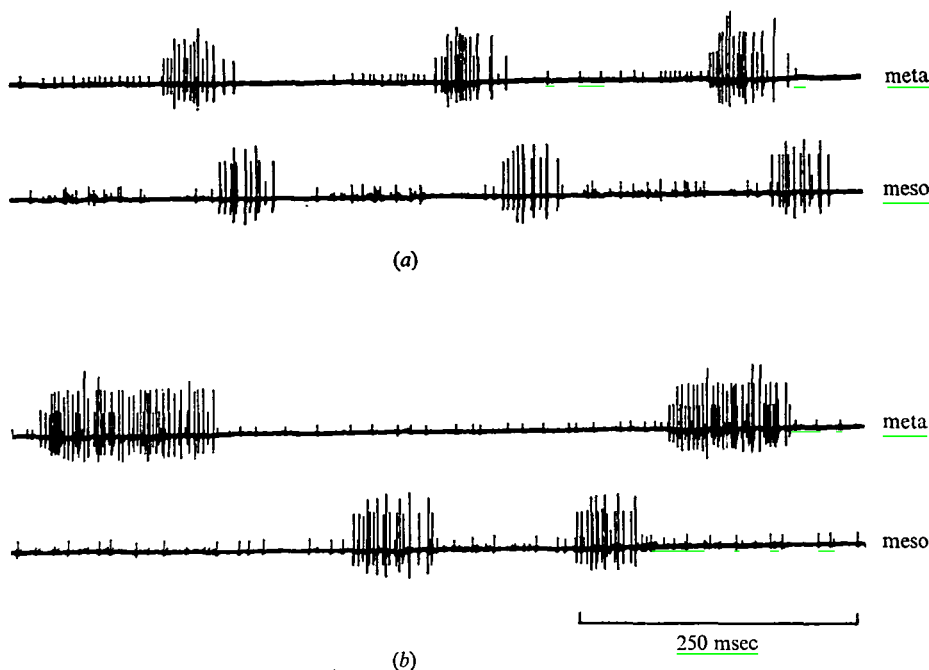
Fig. 3. Burst activity recorded from levator motor axons 5 and 6 in nerve 6Br4 of the meta-thoracic leg (top traces) and the ipsilateral mesothoracic leg (bottom traces) after removal of all sensory input from leg receptors (headless preparation). The two spikes in each burst are from axons 5 and 6 (the spike from axon 6 is the largest) while the smaller spikes are from motor axons 3 and 4 (Pearson & Bergman, 1969). (*a*) Mesothoracic bursts beginning near the end of the metathoracic bursts. (*b*) A metathoracic burst beginning near the end of a mesothoracic burst.

short periods of tripod gait are sometimes seen (ten Cate, 1941). Therefore in both brainless and headless preparations the stepping sequences of ipsilateral meta- and mesothoracic legs are similar to those in intact animals indicating that the head ganglia are of minor importance in co-ordinating movements of the legs.

Following removal of all sensory input from the legs (in both headless and brainless animals) burst activity often persists in motor axons 5 and 6 to the posterior coxal levator muscles of the meso- and metathoracic segments (fig. 3; Pearson & Iles, 1970; Pearson, 1972; Iles 1972). Levator burst generation in the two segments not inde-pendent. Only very rarely do bursts in each segment begin simultaneously and generally there is little overlap of activity. The most common pattern was the meso-thoracic burst beginning immediately after the end of the metathoracic burst (Fig. 3*a*). The reverse, metathoracic following mesothoracic was also fairly common (Fig. 3*b*).

The lack of independence between the bursts generated in the two segments is seen clearly in the histogram of the phase of the mesothoracic bursts in the cycle of the metathoracic bursts (Fig. 4*a*). This phase histogram shows two peaks, whereas none would be expected if the two burst-generating systems were independent. The phase histogram of Fig. 4*a* was constructed from the data from a headless de-afferented animal where the cycle time for the metathoracic bursts (i.e. the time from the begin-ning of one metathoracic burst to the beginning of the next) varied from 100 to 1000 msec. Similar results were obtained in de-afferented brainless preparations. The
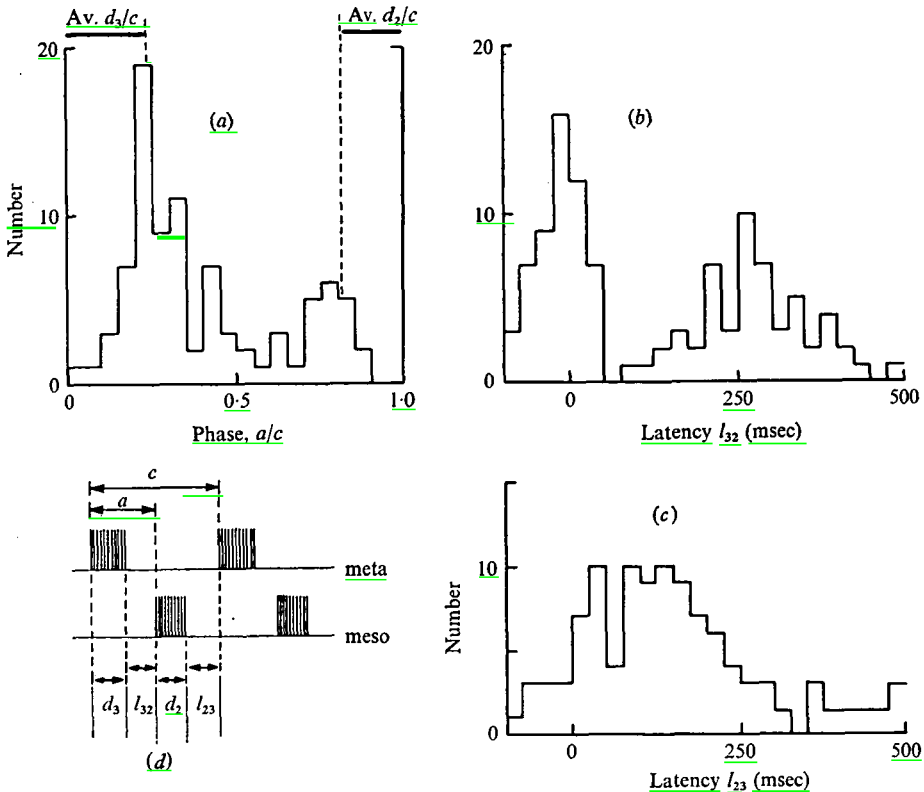
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Fig. 4. (a) Histogram of the phases of mesothoracic bursts in the ipsilateral metathoracic cycle, a/c, observed in a typical preparation after removal of all sensory input from leg receptors. The two peaks in the histogram occur close to the average value of d_3/c and to one minus the average value of d_2/c indicating the tendency for mesothoracic bursts to begin near the end of the metathoracic bursts or vice versa. This tendency is also seen in the latency histograms which show (b) the duration of time between the end of the metathoracic bursts and the beginning of the next mesothoracic burst, l_{32}, and (c) the reverse, l_{23}. Each of these histograms has a prominent peak near zero. (d) illustrates the parameters measured. The histograms were constructed from data obtained from a headless preparation in which the cycle time varied from 100 to 1000 msec.

lengths of the horizontal bars in Fig. 4a plotted from the phase values of 0 and 1 equal the average values of the ratio of metathoracic and mesothoracic burst durations respectively to the cycle time. The ends of these bars correspond to the two peaks in the phase histogram, indicating that the mesothoracic bursts begin near the end of the metathoracic bursts and vice versa. The difference in size of the two peaks is a result of the mesothoracic-following-metathoracic pattern being the most common. The observation that the burst in one segment begins near the end of the burst in the other segment is shown more clearly in histograms of the interval between the end of a burst in one segment and the beginning of a burst in the other segment (Figs. 4b, c). Generally there is less variability in the interval for metathoracic-to-mesothoracic bursts as shown by the sharper peak in the latency histogram.

The distribution of metathoracic and mesothoracic levator burst durations for a headless preparation is shown in Fig. 5. The mean burst duration is smaller in the mesothoracic segment which corresponds to the observation in walking animals that
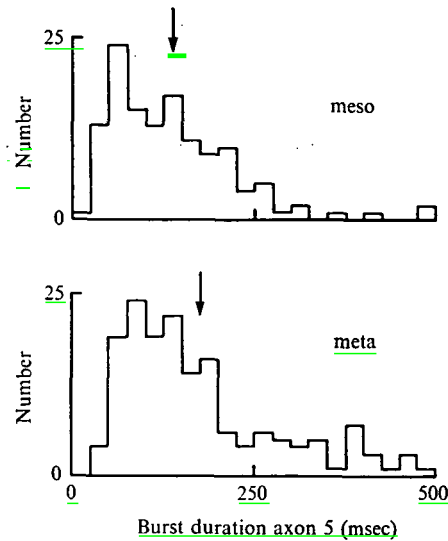
Fig. 5. Histograms of the burst duration of the levator motor axon 5 in nerve 6Br4 of the mesothoracic leg (top) and the ipsilateral metathoracic leg (bottom) recorded in a headless preparation after removal of all sensory input from leg receptors. The arrow above each histogram indicates the average duration of the bursts. The mean duration for the mesothoracic bursts is 136 msec and for the metathoracic is 176 msec.

the protraction time of the mesothoracic leg is shorter (Delcomyn, 1971 *a*), and moreover the magnitudes of these durations are in close agreement with the protraction times observed in walking animals.

(3) *Recordings from connective filaments*

The tendency of levator burst activity in the two segments of de-afferented preparations not to overlap clearly demonstrates that the systems of cells generating these bursts in the two segments must be centrally coupled, and indicates some form of inhibitory coupling between the burst-generating systems. The tendency for the mesothoracic bursts to follow the metathoracic and vice versa suggests that the two burst-generating systems mutually inhibit each other and the following burst is initiated by some form of post-inhibitory rebound process. If there is mutual inhibitory coupling between levator burst-generating systems in the two ganglia then axons should exist in the connectives which discharge in phase with the levator bursts in either the metathoracic or the mesothoracic segment. Axons having these discharge characteristics were found regularly in the dorso-lateral region of the ipsilateral connective (Figs. 6 and 7). Since de-afferentation decreases the occurrence of spontaneous burst activity and the ease with which burst activity can be elicited by stimulation of the cercus (Pearson, 1972), these experiments were performed in animals with the sensory input from leg receptors intact. Because the sensory input was not removed the problem arises as to whether the axons in the connectives whose activity is correlated with the levator bursts are branches of sensory axons or interneurones driven by sensory axons, or whether they are interneurones belonging to the burst-
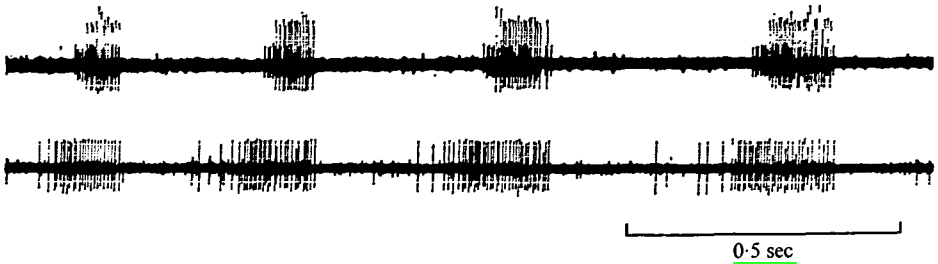
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0·5 sec

Fig. 6. Correlated burst activity of an interneurone in the meta-mesothoracic connective and of levator motor axons 5 and 6 of the ipsilateral metathoracic leg. Top trace, record from nerve 6Br4; bottom trace, record from a small filament teased from the ipsilateral connective after the whole connective had been transected close to the mesothoracic ganglion.
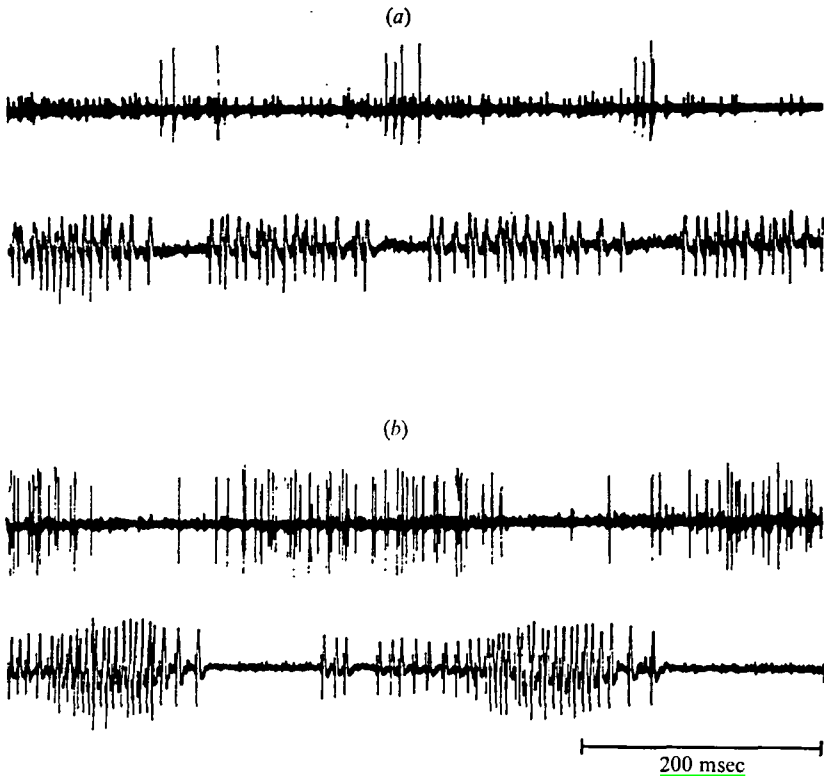
(a)

(b)

200 msec

Fig. 7. Reciprocal burst activity between interneurones in a meso-metathoracic connective and the slow motor axon to the coxal depressor muscle of the ipsilateral mesothoracic leg. Top traces, records from a small filament teased from the connective after it had been transected close to the metathoracic ganglion; bottom traces, junctional potentials recorded extracellularly from the coxal depressor muscle 135D of the ipsilateral mesothoracic leg. Since the slow depressor motor axon discharges reciprocally with the levator axons 5 and 6 (Pearson & Iles, 1970), the burst activity of the interneurones is correlated with bursts of the levator axons 5 and 6 of the mesothoracic leg.

enerating systems or interneurones driven by these systems. A number of observations indicate that these units are not directly activated by sensory input:

(1) They do not discharge during gentle passive movements of the legs.

(2) Pressure to various regions of the legs does not activate these units provided no burst activity is initiated in levator motoneurones.

(3) The discharge characteristics are not noticeably altered by restricting or preventing leg movements, procedures which would be expected to alter sensory input.

The conclusion from these observations is that the axons in the meso-metathoracic connectives discharging in phase with the ipsilateral levator bursts arise from neurones which are part of the levator burst-generating systems or are interneurones driven by these systems.

Axons which could be readily activated by gentle pressure to localized areas of the leg, for example, the pretarsus, were found in the ventral region of the ipsilateral connective. The discharge patterns of these axons suggested they were either collaterals of primary afferent fibres or interneurones strongly excited by primary afferents.

No units were observed discharging in phase with the depressor bursts. This observation further supports the conclusion of an earlier study (Pearson & Iles, 1970) that the locomotory rhythm generator is asymmetric.

(4) *Effect of lesions to the nervous system*

(a) *Connectives*. Although interneurones have been found in the meso-metathoracic connectives which discharge in phase with bursts of activity in levator motoneurones of either the ipsilateral meso- or metathoracic segment (Figs. 6 and 7), it has not yet been established that these interneurones play any part in the co-ordination of stepping in the meso- and metathoracic legs. However, if they do mediate coupling between the two segments then it would be expected that cutting one connective would not prevent co-ordinated movements of the contralateral meso- and metathoracic legs. Furthermore, co-ordinated leg movements might persist following partial transection of those parts of both connectives not containing the interneurones discharging in phase with the levator bursts.

Cutting a single meso-metathoracic connective in *Blatta* does not alter the sequence of leg movements on the contralateral side (Hughes, 1957), although the phase relationships for a given walking speed are changed (compare figures 1 and 10 in Hughes, 1957). Delcomyn (1971*b*) also reported a change in phase in *Periplaneta* but gave no details. In the current investigation the activity in the meso- and metathoracic levator motoneurones 5 and 6 was recorded following transection of the contralateral meso-metathoracic connective. The clear effect is a reduction in the phase of mesothoracic bursts in the metathoracic cycle for all walking speeds slower than 5 steps/sec. The average phase of the mesothoracic burst in the metathoracic cycle in three animals was 0·41, 0·37 and 0·31 for walking speeds of 4, 3 and 2 steps/sec. respectively. These values can be compared with those in Fig. 2. The exact cause of this change in co-ordination is not known (see Discussion) but altered reflex effects in the legs on the unoperated side cannot be excluded, since the orientation of the body is abnormal. The operated side is depressed relative to the unoperated side, and the body is turned markedly towards the unoperated side. Therefore loading conditions in the legs on the

unoperated side are probably quite different from normal, thus altering the magnitud⟨
of any reflexes involved in co-ordinating the leg movements.

The interneurones discharging in phase with the levator bursts were found to be
located in the dorso-lateral region of the connectives. In four animals the medial
halves of both meso-metathoracic connectives were severed. No change from normal
was observed in the stepping movements of the metathoracic and the mesothoracic
legs. On the other hand, cutting the lateral halves of both connectives in two animals
abolished all locomotory movements of the metathoracic legs. From these observations
it can be concluded that the interneurones responsible for co-ordinating stepping
movements of the meso- and metathoracic legs are located in the lateral half of each
connective and, moreover, descending command interneurones activating the loco-
motory rhythm generator in the metathoracic segment must also be laterally located in
the connectives.

(b) *Nerve 3b and* 5. Almost the entire afferent input from sensory receptors in the
legs *Peripheta* reaches the ganglia via nerves 3b and 5 (Dresden & Nijenhuis,
1958; Pipa & Cook, 1959). Afferent fibres in nerve 3b arise mainly from air plates
and chordotal organs in the coxa while those in nerve 5 arise from the campaniform
sensilla in the trochanter and a variety of receptors in the femur, tibia and tarsus.
Nerve 3b can be transected proximal to the coxa with no damage to leg or thoracic
musculature. In three animals this nerve was severed in all six legs and the subsequent
behaviour was observed. Following this operation the animals continued to walk using
the normal tripod gait, but the body was held in an elevated position. Thus severing
nerve 3b does not abolish rhythmic leg movements nor does it prevent the normal
pattern of stepping.

No method was found for severing nerve 5 between branch 5r1 and the trochanter
without damaging muscles in the coxa. Thus the effect of moving virtually all
afferent input via nerve 5 could not be studied. As an alternative, nerve 5, together
with all the muscles in the femur, was transected midway along the femur. A small
hole was made in the cuticle of the femur and the tips of a fine pair of scissors were
inserted to cut nerve 5 and all the muscles. This operation removed all sensory input
from receptors in the tarsus, tibia and the distal end of the femur, and abolished all
active movements of the tibia and tarsus. Therefore, the sensory input remaining in
nerve 5 arose mainly from receptors in the trochanter. Following this operation in the
six legs, all three animals studied readily walked over flat surfaces using a tripod gait.
The flexion and extension movements of the femur persisted since the motor nerves
supplying the muscles giving these movements (nerves 6Br4 and 5r1) were intact.
Thus none of the receptors in the tarsus, tibia or distal part of the femur are essential
for producing the rhythmic movements of the femur during walking, or for co-
ordinating these movements in the different legs.

Although the ability of the animals to walk over smooth surfaces was not markedly
affected by either cutting nerve 3b in all six legs, or cutting nerve 5 and femoral
muscles midway along the femur in all six legs, a combination of these two sets of
operations drastically diminished the animal's ability to walk. Following the combined
operation the animals had a very abnormal posture due to hyperactivity in the remotor
muscles of the coxa and the coxal depressor muscles. Thus the animals stood with the
body markedly elevated from the ground. When induced to walk these animals fell

requently and often turned over on the back, a position from which they were unable to recover. However, occasionally falling did not occur and the animals walked a short distance displaying an apparently normal tripod gait. The exact phase relations between stepping of the different legs were not measured in any of these animals but it was very clear from visual observation that the steps of the ipsilateral meso- and metathoracic legs alternated. An additional observation in these animals was that rapid running movements could not be elicited and the occurrence of spontaneous locomotory movements was markedly reduced.

(5) *Effects of amputating and restraining the mesothoracic legs*

Following amputation of the mesothoracic legs close to the trochanter there is an immediate change in the phase of the movements of the remaining meta- and prothoracic legs (Hughes, 1957; Wilson, 1966; Delcomyn, 1971 b). In normal animals a prothoracic leg steps at approximately the same as the ipsilateral metathoracic leg, whereas after removal of the mesothoracic legs at the trochanter the prothoracic legs step shortly after the ipsilateral metathoracic legs in slowly walking animals (Delcomyn, 1971 b). To measure this change in co-ordination by electrophysiological methods the activity in the slow motoneurone to the coxal depressor muscles of the pro- and metathoracic legs on one side was recorded before and after amputation of the mesothoracic leg. In these animals the femur was transected just distal to the trochanter. The effect of amputation is shown graphically in Fig. 8. The phase of the prothoracic step in the metathoracic cycle was slightly less than $1 \cdot 0$ for normal animals. In femoral amputees there is a dramatic decrease in the phase for a given cycle time and, corresponding to the behavioral observations, there is an increase in phase with an increase in the speed of walking.

A possible explanation for the change in co-ordination is that it is simply due to changes in the magnitude of intrasegmental reflexes in the remaining pro- and metathoracic legs resulting from an alteration in the load carried by these legs. If this wholly explained the change in co-ordination then a similar result would be expected if the intact mesothoracic legs were tied to the thorax and prevented from touching the ground. The mesothoracic legs were fixed to the thorax by passing a fine thread through each mesothoracic femur and binding the femur close to a small metal ring attached to the animal's thorax. Each femur then lay along the lateral part of the thorax. In addition to keeping the mesothoracic legs off the ground, tying the legs in this manner prevented rhythmic movements of the femur and tibia. The very interesting observation in these animals was that there appeared to be a complete loss of co-ordination between the prothoracic and metathoracic legs, rather than a change in the pattern of co-ordination similar to that occurring after amputation of the mesothoracic legs at the trochanter. The animals had extreme difficulty in walking and no clear phase relationship of stepping of the prothoracic legs in the metathoracic cycle was observed. This observation is analogous to the findings in stick insects where it has been reported that prevention of stepping of the mesothoracic legs also results in a loss of co-ordination between the pro- and metathoracic legs (Wendler, 1966).

Another method used for fixing the intact mesothoracic legs so as to prevent them from touching the ground was to tie the lateral edge of each coxa to the thorax. Unlike the previous method this did not prevent rhythmic movements of the femur and tibia
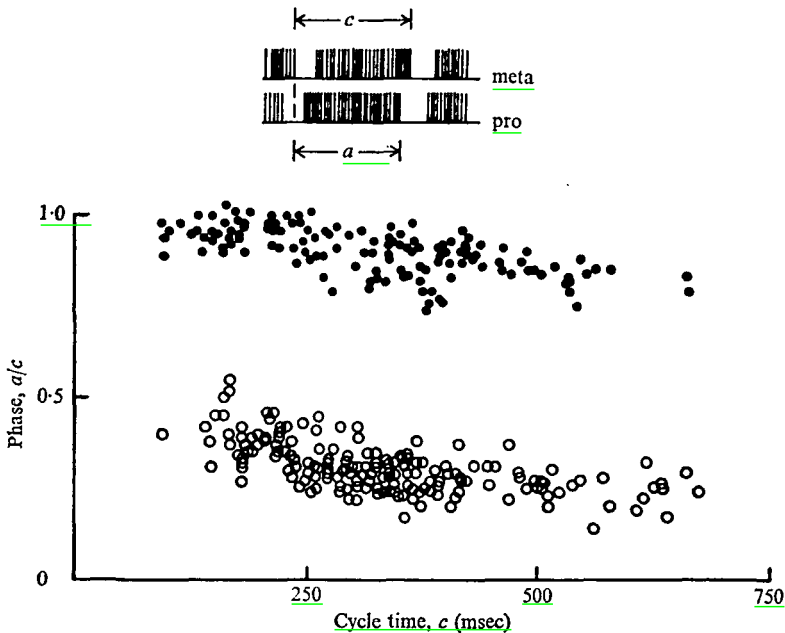
Fig. 8. Phase of the prothoracic levator bursts in the cycle of the ipsilateral metathoracic levator bursts, a/c, as a function of cycle time, c. Filled circles, intact walking animal; open circles, after amputation of both mesothoracic legs at the trochanter. In these experiments the activity in the single slow motor axon to the coxal depressor muscles of each leg was recorded. Levation occurs during the silent periods in these records. The two parameters measured are shown in the diagram at the top of the figure.

during walking. The result of fixing the mesothoracic legs in this manner was that the prothoracic and metathoracic legs stepped with exactly the same pattern as in femoral amputees. During walking in these animals the femurs of the tied mesothoracic legs moved rhythmically, and during slow walking there were two flexion movements of the femur for each step of the ipsilateral metathoracic leg. One of the main conclusions from these experiments is that the change in co-ordination after mesothoracic leg amputation at the trochanter is due to an alteration in the sensory input from the mesothoracic legs, and not due to changes in loading of the remaining pro- and metathoracic legs.

To determine which receptors were involved in causing this change in co-ordination, lesions were made to the nervous system and muscles. No change from normal in the co-ordination of the pro- and metathoracic legs was observed in animals in which nerve 3b had been cut in both mesothoracic legs and the mesothoracic legs had been amputated just proximal to the tibia. Delcomyn (1971b) also reported a normal tripod gait in tibial amputees. Thus the receptors responsible for causing the change in co-ordination must be located in the trochanter and/or the proximal end of the femur. In animals with the mesothoracic legs amputated at the distal end of the femur the end of the femur is pressed against the ground during walking and it would therefore be expected that the campaniform sensilla of the trochanter would be excited during leg extension. On the other hand, when the femur is cut close to the trochanter no part of the remaining mesothoracic legs usually touched the ground, and the stepping
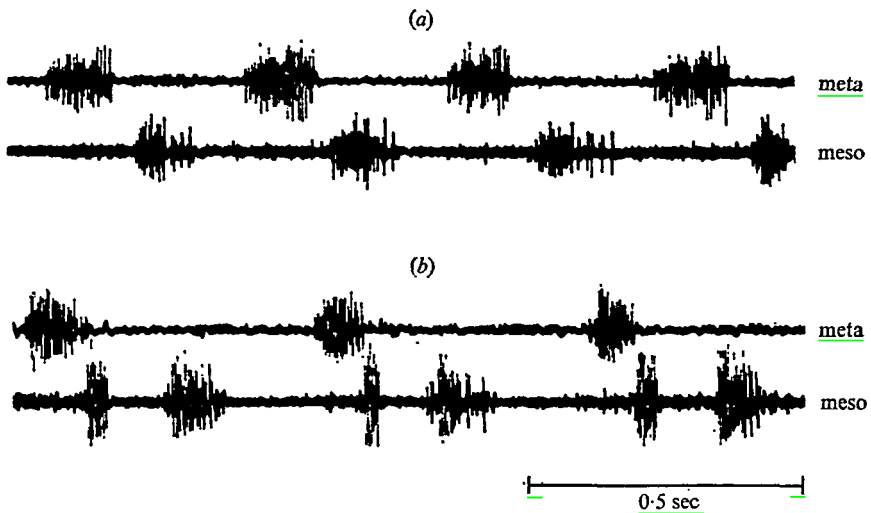
(a)

meta

meso

(b)

meta

meso

0·5 sec

Fig. 9. Burst activity recorded from the posterior coxal levator muscles of the metathoracic leg (top traces) and the ipsilateral mesothoracic leg (bottom traces) in a walking animal after cutting nerve 3b in both mesothoracic legs and amputation of both mesothoracic legs at (a) the distal end of the femur, and (b) the proximal end of the femur.

of the remaining legs is altered. It is unlikely that this change in co-ordination is due to removal of additional receptors in the femur because destruction of the group of campaniform sensilla in the proximal end of the femur (Pringle, 1938) did not change co-ordination in animals with their mesothoracic legs amputated just proximal to the tibia, and furthermore, no other important group of active receptors is removed by transecting the mesothoracic legs through the proximal ends of the femurs as compared to transecting them at the distal ends. When the amputated mesothoracic legs do not touch the ground the trochanteral campaniform sensilla would not be excited because the cuticle of the trochanter would not be strained. Thus the alteration of co-ordination following amputation of the mesothoracic legs close to each trochanter is most prob-ably due to a reduction in the sensory input from the trochanteral campaniform sensilla.

An important correlation was observed between the discharge patterns of the meso-thoracic coxal levator motoneurones and the pattern of co-ordination in the pro- and metathoracic legs. Fig. 9a shows an animal with the mesothoracic femur severed just proximal to the tibia, and it can be seen that there is a clear alternation between burst activity in the mesothoracic and metathoracic segments. As already mentioned, under this condition there is a normal tripod gait. When the femur was severed close to the trochanter, altering the co-ordination of stepping in the prothoracic and metathoracic legs, double burst activity was observed in the mesothoracic coxal levator moto-neurones for each burst in the metathoracic segment (Fig. 9b). The first mesothoracic burst began immediately after the cessation of each metathoracic burst, a character-istic which is similar to that occurring in de-afferented preparations (Fig. 3). This first burst was always the most intense and the shorter in duration.
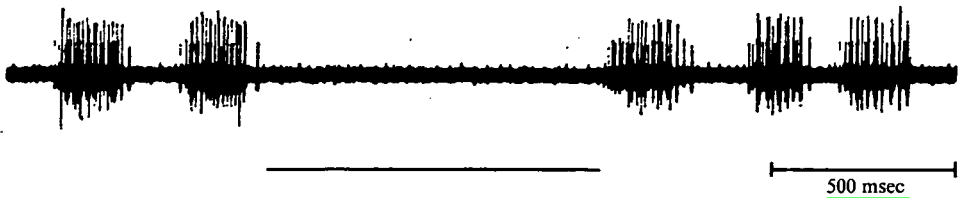
Fig. 10. Inhibition of burst activity in levator motor axons 5 and 6 by stimulation of the trochanteral campaniform sensilla. Light pressure was applied to the trochanter during the interval indicated by the horizontal bar. The record is from nerve 6Br4 of the metathoracic leg after transection of the connectives between the mesothoracic and metathoracic ganglia.

(6) Reflexes from trochanteral receptors

Since receptors in the trochanter and proximal part of the femur appear to be of considerable importance in co-ordinating leg movements during walking, it is necessary to establish the reflex effects initiated by activity in these receptors. Previously it has been shown that stimulation of the most prominent group of receptors in the trochanter, the campaniform sensilla, excites the slow motoneurone to the coxal depressor muscles (Pearson, 1972; Pringle, 1940). In addition it has been proposed that these receptors inhibit a bursting interneurone (or system of interneurones) which excites the levator motoneurones (Pearson, 1972). To obtain more direct evidence for this inhibitory reflex pathway, the trochanter of a metathoracic leg was gently squeezed during periods of spontaneous burst activity in the levator motor axons 5 and 6 in nerve 6Br4 of the same leg. The meso-metathoracic connectives were severed because rhythmic burst activity occurs more readily in such preparations. The obvious effect of gentle pressure on the trochanter was a cessation of burst activity in the levator motoneurones, the inhibition lasting as long as the pressure was applied (Fig. 10). Burst activity often continued immediately after removal of the stimulus, but the time of initiation of the first burst following the stimulus was not necessarily at the instant when a burst of activity would have been expected had not the trochanter been stimulated. The stimulus apparently inhibits the system of interneurones primarily responsible for producing the levator burst activity rather than simply inhibiting the motoneurones which are driven by this system.

DISCUSSION
(1) Central coupling

The problem of how the movements of the different legs of insects are co-ordinated during walking has been discussed in a number of previous publications (Hughes, 1952, 1957; Wilson, 1966, 1967; Pringle, 1961; Delcomyn, 1971b; Wendler, 1966). From these discussions it emerges that at least three mechanisms could play a role in co-ordinating the leg movements. First, co-ordination may depend on central connexions between segments whose properties are independent of any sensory input. Secondly, intersegmental reflex pathways may function so that the motor output in any one leg depends on the phasic sensory input coming from other segments. Finally, there may be indirect coupling of intrasegmental reflexes via a mechanical linkage such that movements of any one leg alter the sensory input in other legs and thereby

Indirectly control the movements of the other legs. Until now the latter two of these possibilities have seemed improbable (Delcomyn, 1971 c; Wilson, 1966; Wendler, 1966) and yet the evidence for central coupling has been weak (Wilson, 1966). One of the clear results of the current investigation is that after removal of all sensory input from the legs the burst activity in levator motoneurones of the ipsilateral meso-thoracic and metathoracic legs is negatively correlated (Figs. 3, 4). This is the most compelling evidence so far for the existence of central coupling. It seems very unlikely that these patterns of burst activity are related to any behaviour other than walking because rhythmic movements of the mesothoracic legs occur only during locomotion or attempted locomotion. Furthermore, the levator burst durations in the meso-thoracic segment are on average shorter than those in the metathoracic segment (Fig. 5) which corresponds to the behavioural observation of a shorter protraction time of the mesothoracic legs during walking (Delcomyn, 1971 a). The magnitude of the durations of the bursts in both segments is also consistent with the proposal that these bursts are related to locomotory behaviour. However, not all aspects of the burst patterns recorded in de-afferented preparations correspond to those observed in the walking animals (compare Figs. 1 and 3). The most noticeable difference is that in de-afferented animals the mesothoracic burst begins immediately after the end of the metathoracic burst or vice versa, whereas in walking animals there is an obvious latency which depends on the walking speed (Fig. 2). This marked difference indicates that the leg movements are not entirely centrally co-ordinated and must depend to some extent on sensory input. The question of how sensory input may modify the central coupling is considered in § 3 of this discussion.

The observation of little overlap of the mesothoracic and metathoracic levator bursts in de-afferented preparations, together with the finding of two peaks in the phase histogram (Fig. 4a), suggested that the system of cells in each half-ganglion generating these bursts mutually inhibit each other. A search for axons in the meso-metathoracic connectives which could be mediating this mutual inhibition was successful (Figs. 6, 7). Although these interneurones discharge in phase with the levator bursts there is as yet nothing to indicate that they do inhibit the burst-generating system in the adjacent segment. All that can be said at this stage is that a necessary condition for mutual inhibition between segments is the existence of such interneurones, a condition which is fulfilled. The possible importance of these interneurones in co-ordinating leg movements was strengthened by the observation that severing the lateral halves of the meso-metathoracic connectives (where the interneurones were found), abolished all locomotory movements of the metathoracic legs, whereas severance of the medial halves did not alter the normal tripod gait.

(2) *Sensory influences in co-ordination*

We turn now to consider which receptors are important in controlling the co-ordination of leg movements. The only group of receptors which could be essential for co-ordinating the leg movements must be located in the trochanter. This conclusion follows from the observation that the tripod gait is often observed in animals after severance of nerve 5 in the femur and nerve 3b proximal to the coxa in all six legs. After these lesions the only significant group of leg receptors still connected to the ganglion is the campaniform sensilla of the trochanter. In addition to this observa-

tion the importance of the trochanteral campaniform sensilla is strongly indicated by the finding that, after cutting nerve 3b in both mesothoracic legs, amputation of these legs just proximal to the tibia did not alter the normal phase relations of stepping in the prothoracic and metathoracic legs, whereas severance of the mesothoracic legs close to the trochanter (which did not remove any further groups of active receptors) altered the gait in the remaining legs. The major difference between these two pre-parations is that in the former the distal end of the femur touches the ground and participates in the locomotory behaviour, whereas in the latter it does not. Thus in the former the campaniform sensilla of the trochanter are probably excited to a far greater extent than in the latter. The conclusion from these observations is that receptors located in the trochanter, most probably the campaniform sensilla, are of considerable importance in co-ordinating leg movements. Input from these receptors has been shown to have a strong excitatory effect on the slow motoneurone to the coxal depressor muscles of the same leg (Pringle, 1940; Pearson, 1972) and a strong inhibitory effect on the system of neurones generating the levator bursts in the same leg (Fig. 10). An important consideration is whether the input from these receptors also influences the motor output in adjacent segments. Preliminary experiments have failed to reveal any intersegmental effects arising from an increase in activity in the trochanteral campaniform sensilla. Pringle (1940) also failed to find intersegmental reflex effects on stimulation of these receptors. Thus the intersegmental reflex effects described in previous publications (Wilson, 1965; Delcomyn, 1971c) probably arise from receptors other than the trochanteral campaniform sensilla. Since these other receptors appear to be unimportant in co-ordinating leg movements it may be concluded that direct intersegmental reflex pathways are of little or no importance in the co-ordination of leg movements.

(3) Model

A hypothetical scheme for describing the generation of reciprocal activity patterns in levator and depressor motoneurones of a single segment, and the timing of levator bursts in ipsilateral segments is shown in Fig. 11. The proposed mechanism for producing reciprocal burst activity within a single half segment has been described in detail in previous papers (Pearson & Iles, 1970; Pearson, 1972). A bursting inter-neurone, b.i. (or group of bursting interneurones) is postulated to excite the levator motoneurones and inhibit the ongoing activity in depressor motoneurones. The rate of bursting is assumed to depend on the level of activity in descending command interneurones, an increase in command input producing an increase in the burst rate. In the earlier study the evidence for an inhibitory pathway from the campaniform sensilla to the bursting interneurone was indirect. Direct evidence for the existence of such a pathway has now been obtained by observing a cessation of burst activity on stimulation of the campaniform sensilla (Fig. 10). The onset of burst activity following stimulation did not necessarily correspond to the point where burst activity would have been expected had the campaniform sensilla not been stimulated. Thus the campaniform sensilla inhibit the system of cells producing the bursts in the levator motoneurones and not simply the levator motoneurones. This result, however, does not mean that direct inhibitory pathways from the campaniform sensilla to the levator motoneurones do not also exist.

To describe the co-ordination of levator burst activity in adjacent ipsilateral legs
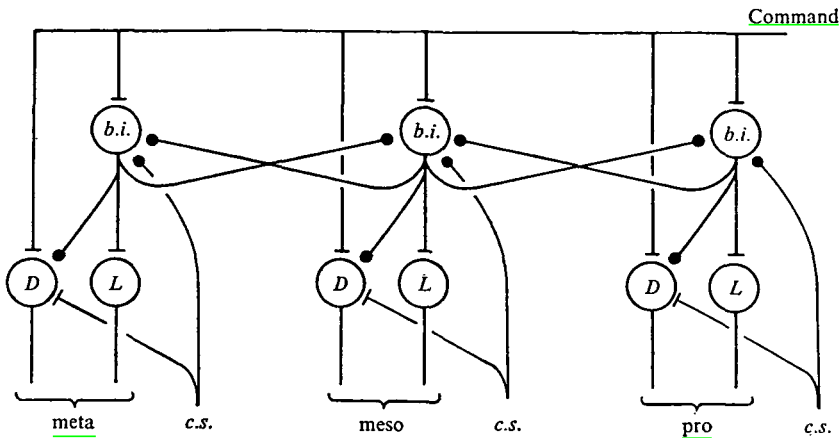
Fig. 11. Hypothetical scheme for describing the patterning of activity in the coxal levator and depressor motoneurones of the homolateral legs during walking. Filled endings, inhibition; bar endings, excitation; *b.i.*, bursting interneurone; *c.s.*, campaniform sensilla; *D*, depressor motoneurones; *L*, levator motoneurones. See text for details.

it is proposed that the bursting interneurones in adjacent ipsilateral segments mutually inhibit each other. In Fig. 11 these inhibitory pathways are shown as collaterals of the bursting interneurones, but it is obvious that more complex systems could exist.

At present we have no data concerning the mechanisms co-ordinating stepping of contralateral legs of the same segment. Previously it has been assumed that the systems producing locomotory leg movements in contralateral legs mutually inhibit each other (Hughes, 1957; Wilson, 1967) but results from de-afferented preparations have failed to demonstrate any central coupling between levator burst-generating systems on opposite sides of the same segment (Iles, 1972). Reflex interaction between the two legs of one segment are fairly strong (Pringle, 1940; Wilson, 1965) and it is possible that this is sufficient to co-ordinate the legs of the same segment. However, the possibility of central inhibitory coupling cannot be excluded for the efficacy of this coupling may depend on activity in descending command pathways which was absent in experiments on de-afferented preparations.

A major problem arising from observations in de-afferented and in walking animals is to explain the difference between the patterns of burst activity in the mesothoracic and metathoracic levator motoneurones in these two preparations. In de-afferented animals the mesothoracic levator bursts tend to begin immediately after the meta-thoracic levator burst and vice versa (Figs. 3, 4), whereas in walking animals there is an obvious delay between the end of a burst in one segment and the beginning of a burst in the other segment (Fig. 2). It is proposed that this difference can be explained by the existence of the inhibitory reflex pathways from the campaniform sensilla to the bursting interneurones. In de-afferented preparations this inhibitory pathway is absent and one possibility is that following a burst in one segment there is a tendency for a burst to be initiated in the adjacent segment by post-inhibitory rebound excitation. In a walking animal, however, there will be inhibition of the bursting inter-neurone from the campaniform sensilla during and following stepping in the adjacent ipsilateral leg, and it is therefore proposed that this inhibition is sufficient to suppress

the immediate initiation of burst activity by post-inhibitory rebound. Support for this proposal comes from observations in animals after amputation of the mesothoracic legs. Amputation close to the trochanter reduces the input from the campaniform sensilla and leads to double burst activity in the mesothoracic levator motoneurones, the first of these bursts occurring immediately after the termination of the meta-thoracic burst (Fig. 9). In these preparations the prothoracic leg steps soon after the metathoracic so the second burst could be initiated by rebound following the pro-thoracic levator burst.

Another important problem to consider is why the co-ordination of the metathoracic and prothoracic legs changes when the mesothoracic legs are amputated close to the trochanter. The most probable explanation is that it is due to a change in the central coupling between the prothoracic and metathoracic ganglia as a result of removal of inhibition from the campaniform sensilla to the bursting interneurone in each half of the mesothoracic ganglion. The change in co-ordination after amputation of the meso-thoracic legs is associated with an altered pattern of burst activity in the mesothoracic levator motoneurones (Fig. 9). Since the bursting interneurones of the prothoracic and metathoracic segments are postulated to be centrally coupled via the bursting inter-neurone of the mesothoracic segment (Fig. 11), any change in the mesothoracic segment would be expected to alter the relative timing of levator burst activity in the prothoracic and metathoracic legs. Although precise measurements have not yet been made it appears that in amputee animals the prothoracic levator bursts are initiated soon after the first of the two mesothoracic bursts.

A further observation explained by the model of Fig. 11 is that tying the intact mesothoracic legs to the thorax in a flexed position results in the abolition of any co-ordinated movements of the prothoracic and metathoracic legs. Under this condition no burst activity is produced in the mesothoracic levator motoneurones due to a maintained inhibitory input from the campaniform sensilla of the trochanter which are highly excited when the leg is fixed in this position (Pringle, 1940). On the model proposed in Fig. 11 the abolition of all activity in the bursting interneurone of the mesothoracic leg would lead to a complete decoupling of the activity in the bursting interneurones of the prothoracic and metathoracic legs. On the other hand when the coxae of both mesothoracic legs are tied to the thorax so that movements of the femur and tibia are not restricted, the movements of the other four legs are similar to those seen in animals after amputation of the mesothoracic legs at the trochanter. In this case the input from the campaniform sensilla of the mesothoracic legs is considerably reduced and double burst activity occurs in the mesothoracic levator motoneurones (indicated by two flexion movements of the mesothoracic femurs for each step of the metathoracic leg). Again, since the bursting interneurones in the ipsilateral prothoracic and metathoracic legs are considered to be coupled via the bursting interneurone of the ipsilateral mesothoracic leg, any change in the bursting pattern in the meso-thoracic interneurone will alter the pattern of stepping in the prothoracic and meta-thoracic legs. Since tying the mesothoracic coxae to the thorax and amputation of the mesothoracic legs just distal to the trochanter both reduce the input from the tro-chanteral campaniform sensilla, it is not surprising that the new stepping patterns produced are similar.

Finally the importance of the inhibitory input from the campaniform sensilla to the

ursting interneurones in controlling the co-ordination of leg movements during walking must be stressed. When an animal is walking slowly the load carried by each leg while in contact with the ground will vary from moment to moment. If the load carried by a leg decreases below a certain level then the inhibition to the bursting interneurone controlling the stepping movement of that leg will be insufficient to prevent the initiation of a burst in that interneurone. Thus the leg will step and the residual load carried by that leg will be distributed amongst the other legs, thus slightly increasing the load carried by these legs and hence decreasing the probability of stepping in them because of an increase in inhibition to their bursting interneurones. Another leg may begin stepping during the step of the first leg since the load carried by the second leg may decrease due to a change in position as the animal moves forward. However, the mutual inhibitory coupling between bursting interneurones in adjacent segments ensures that the second stepping leg is not adjacent to the first. When the stepping leg makes contact with the ground the load carried by the other legs, particularly adjacent legs, will decrease and these may step immediately or shortly after depending on their exact position. Since the stepping movements of any one leg in part depend on the movements of the other legs it is not surprising that complex patterns of leg movements can be observed in animals walking slowly over uneven surfaces. The existence of inhibitory reflex pathways to the bursting interneurone in each half-segment provides an ingenious mechanism for allowing the animal to traverse uneven and unpredictable terrains in a stable manner.

As the walking speed increases, the reflex effects from the campaniform sensilla decrease (Pearson, 1972). Thus in running animals the role of the campaniform sensilla in co-ordinating leg movements is probably insignificant. Co-ordinated movements will then be entirely dependent on the central inhibitory connexions between adjacent segments. It is of interest to note that animals only run over even surfaces where the need for reflex regulation is minimal.

In summary then it is proposed that two major factors are important in controlling leg movements in slowly walking animals. The first of these is mutual inhibitory connexion between adjacent segments, and the second is the indirect coupling of the intrasegmental inhibitory reflex from the trochanteral campaniform sensilla to the bursting interneurone via a mechanical linkage. As the walking speed increases it is proposed that the importance of the second of these factors diminishes leaving purely central control in rapidly running animals. It is considered that direct intersegmental reflexes are of little or no importance in co-ordinating leg movements during walking.

SUMMARY

1. The activity in identical motoneurones innervating leg muscles of the three thoracic segments of the cockroach has been recorded in (a) normal walking animals, (b) walking animals after lesions to the nervous system and/or amputation of the mesothoracic legs, and (c) restrained de-afferented preparations.

2. The phase of levator motoneurone burst activity of the mesothoracic leg in the metathoracic cycle is almost 0.5 for all walking speeds above 2 steps/sec, confirming that a tripod gait is used at all but the slowest speeds.

3. The burst-generating systems in each segment are centrally coupled because in

de-afferented preparations there is a tendency for the bursts in the mesothoracic segment to begin near the end of the metathoracic bursts, and vice versa.

4. Sensory input from leg receptors is also important in co-ordinating stepping movements of the different legs since (a) there are some differences in motoneurone activity of de-afferented and walking preparations, and (b) amputation of the meso-thoracic legs at the trochanter leads to an immediate change in the co-ordination of the remaining four legs.

5. It is proposed that two mechanisms are important in co-ordinating leg move-ments in a slow walking cockroach (a) mutual inhibition between levator burst-generating systems in adjacent ipsilateral legs, and (b) an inhibitory reflex pathway from the campaniform sensilla of the trochanter to the burst-generating system of each leg. The second of these two mechanisms may become less important as the walking speed increases.

REFERENCES

CARBONELL, C. S. (1947). The thoracic muscles of the cockroach, *Periplaneta americana*. *Smithson. misc. Collns* **107**, 1–23.

TEN CATE, J. (1941). Quelques remarques à propos de l'innervation des mouvements locomotaires de la blatte (*Periplaneta americana*). *Archs néer. Physiol.* **25**, 401–9.

DELCOMYN, F. (1971a). The locomotion of the cockroach *Periplaneta americana*. *J. exp. Biol.* **54**, 443–52.

DELCOMYN, F. (1971b). The effect of limb amputation on locomotion in the cockroach *Periplaneta americana*. *J. exp. Biol.* **54**, 453–72.

DELCOMYN, F. (1971c). Computer aided analysis of a locomotor leg reflex in the cockroach, *Periplaneta americana*. *Z. vergl. Physiol.* **74**, 427–45.

DRESDEN, D. & NIJENHUIS, E. D. (1958). Fibre analysis of nerves of the second thoracic leg in *Periplaneta americana*. *Proc. K. ned. Akad. Wet.* C **61**, 213–23.

HUGHES, G. M. (1952). The coordination of insect movements. I. Walking movements of insects. *J. exp. Biol.* **29**, 267–84.

HUGHES, G. M. (1957). The coordination of insect movements. II. The effect of limb amputation and the cutting of commissures in the cockroach (*Blatta orientalis*). *J. exp. Biol.* **34**, 306–33.

ILES, J. F. (1972). Electrophysiological investigations of insect nerves and muscle. D.Phil. Thesis, Oxford University.

PEARSON, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173–93.

PEARSON, K. G. & BERGMAN, S. J. (1969). Common inhibitory motoneurones in insects. *J. exp. Biol.* **50**, 445–73.

PEARSON, K. G. & ILES, J. F. (1970). Discharge patterns of coxal levator and depressor motoneurones in the cockroach *Periplaneta americana*. *J. exp. Biol.* **52**, 139–65.

PEARSON, K. G. & ILES, J. F. (1971). Innervation of the coxal depressor muscles of the cockroach, *Periplaneta americana*. *J. exp. Biol.* **54**, 215–32.

PIPA, R. L. & COOK, E. F. (1959). Studies on the hexapod nervous system. I. The peripheral distribu-tion of the thoracic nerves of the adult cockroach, *Periplaneta americana*. *Ann. ent. Soc. Am.* **52**, 695–710.

PRINGLE, J. W. S. (1938). Proprioception in insects. II. The action of the campaniform sensilla on the legs. *J. exp. Biol.* **15**, 114–31.

PRINGLE, J. W. S. (1940). The reflex mechanism of the insect leg. *J. exp. Biol.* **17**, 8–17.

PRINGLE, J. W. S. (1961). Proprioception in arthropods. In *The Cell of the Organism* (ed. J. A. Ramsay and V. B. Wigglesworth). Cambridge.

ROEDER, K. D. (1937). The control of tonus and locomotor activity in the praying mantis (*Mantis religiosa* L.). *J. exp. Zool* **76**, 353–74.

WENDLER, G. (1966). The coordination of walking movements in arthropods. *Symp. Soc. exp. Biol.* **20**, 229–50.

WILSON, D. M. (1965). Proprioceptive leg reflexes in cockroaches. *J. exp. Biol.* **43**, 397–409.

WILSON, D. M. (1966). Insect walking. *A. Rev. Ent.* **11**, 103–22.

WILSON, D. M. (1967). An approach to the problem of control of rhythmic behaviour. In *Invertebrate Nervous Systems* (ed. C. A. G. Wiersma), pp. 219–30. Chicago.