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DISCHARGE PATTERNS OF COXAL LEVATOR AND DEPRESSOR MOTONEURONES OF THE COCKROACH, *PERIPLANETA AMERICANA*

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INTRODUCTION

The control of rhythmic behaviour has been intensively studied in a number of arthropod systems, namely insect flight (Wilson, 1961, 1968; Wyman, 1965, 1966), insect ventilation (Miller, 1965, 1966; Mill & Hughes, 1966) and crayfish and lobster swimmeret (Hughes & Wiersma, 1960; Davis, 1968, 1969). The patterning of motoneuronal activity in these systems is to a large extent independent of sensory feed back, depending primarily upon the properties and connectivity of cells within the central nervous system. In contrast to these systems the control of rhythmic leg movements during insect walking is poorly understood and there is currently no information as to the extent of central patterning of activity in leg motoneurones.

Recent studies on the locust have shown that sensory input from the femoral chordotonal organ and tarsal hairs is important in co-ordinating leg movements and controlling excitatory and inhibitory motoneuronal activity when the animal is making postural adjustments and walking (Usherwood, Runion & Campbell, 1968; Runion & Usherwood, 1968). These studies have therefore suggested, for the locust at least, that sensory input from leg receptors is very important for controlling motoneuronal activity during walking. Currently there is no direct evidence to indicate that there is any significant central patterning of motoneuronal activity in locust walking. Investigations on the cockroach, *Periplaneta americana*, have also shown that sensory input could be important in patterning activity in motoneurones of a single leg, and for co-ordinating the activity in motoneurones in different legs (Pringle, 1940, 1961; Wilson, 1965). However, in this animal there is indirect evidence suggesting that during walking motoneuronal activity may be patterned independent of sensory input (Milburn, 1963; Wilson, 1966*a*).

The aim of the present investigation has been to determine the extent of central patterning of activity in motoneurones innervating some of the metathoracic coxal levator and depressor muscles by recording the activity of identifiable motoneurones in preparations in which all sensory input from leg receptors has been eliminated.

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PREPARATIONS AND METHODS

1. *Anatomy*

The positions of the nerves studied in this investigation are shown in Fig. 1. The metathoracic posterior coxal levator muscle (number 182, notation of Carbonell, 1947) is innervated by axons contained in nerve 6Br4 (notation of Pipa & Cook, 1959). There are twelve motor axons in nerve 6Br4. These axons have been numbered from 1 to 12 with increasing size (Pearson, Stein & Malhotra, 1969). Axons 3, 4, 5 and 6 innervate the posterior coxal levator muscles 182C and 182D. The innervation patterns of these muscles by axons 3–6 have been described by Pearson & Bergman (1969). Axon 3 is one branch of a common inhibitory neurone having a powerful inhibitory effect on the slow contractions produced by axon 4. Activity in axons 5 and 6 produces strong tonic contractions of the posterior coxal levator muscles (182C,D) (Fig. 7), which are much less affected by the common inhibitory neurone.

The coxal depressor muscles (177D, E, 178 and 179) are innervated by motor axons contained in nerve 5r1. Nerve 5r1 arises soon after nerve 5 enters the coxa and runs only a short distance before branching, branch 5r1a running ventrally and 5r1b running dorsally.

2. *Preparations*

All experiments were carried out on the right side of the metathoracic segment of adult male cockroaches, *Periplaneta americana*. Animals were lightly anaesthetized with carbon dioxide and pinned ventral side up to a cork board. One pin was placed

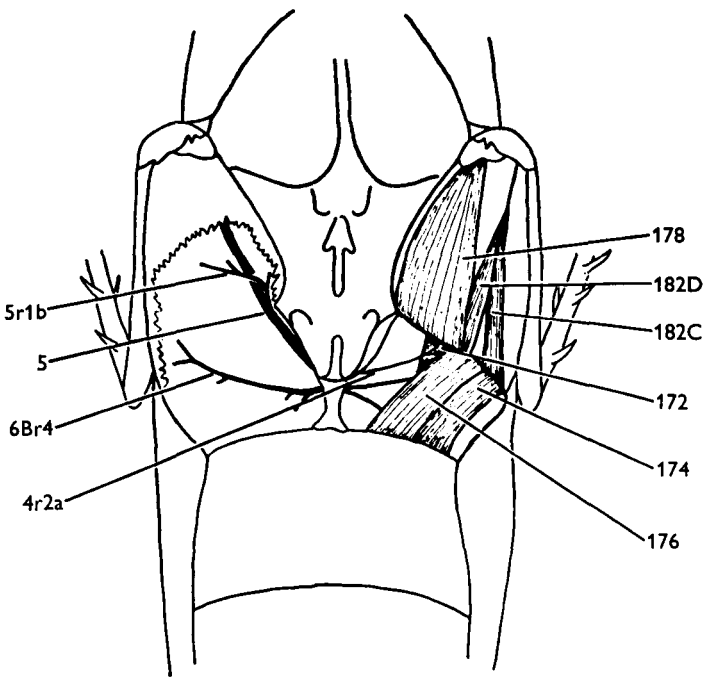

Fig. 1. Diagram showing the position of the nerves and muscles relevant to the investigation on the discharge patterns of the coxal levator and depressor motoneurones. Both metathoracic legs have been rotated to expose their dorsal coxal surfaces.

off centre near the last abdominal segment and another through the dorsal cuticle just anterior to the prothoracic segment. The right metathoracic coxa was rotated to expose its dorsal surface and fixed with a fine pin mid-way along the medial cuticle. Care was taken to ensure that the femur, tibia and tarsus of this leg were completely free and able to move to their full extent. The prothoracic and mesothoracic legs were therefore pulled forward and fixed so as not to interfere with any part of the rotated metathoracic leg. The metathoracic leg not being studied was pinned ventral side up, the pin passing through the lateral edge of the coxa. Again care was taken to ensure that the femur, tibia and tarsus of this leg were completely free.

The dissection to expose nerve 6Br4 has been described elsewhere (Pearson & Bergman, 1969). To expose nerve 5r1 the coxa was first rotated to reveal its dorsal surface. The dorsal coxal rim was cut both sides of the attachment of muscle 178 and the dorsal coxal cuticle broken in an arc between these two cuts. This arc extended about two-thirds the length of the coxa. Muscle 178 was detached from this small piece of cuticle. By moving the detached muscle 178 laterally, and carefully removing the underlying tracheal system, nerve 5r1 b was exposed. A fine branch of this nerve is sent to muscle 178. The approximate position of nerve 5r1 b is shown in Fig. 1 (muscle 178 is not shown connected to the nerve in this diagram). By moving nerve 5 laterally nerve 5r1 a is exposed. This dissection allowed recordings to be made from nerve 5r1, 5r1 a or 5r1 b.

(*a*) *Headless preparation.* The head was removed before pinning the animal to the cork board and nerves 6Br4 and 5r1 were exposed as described above. No peripheral nerves were cut in this preparation.

(*b*) *De-afferentated preparation.* This preparation was designed to eliminate all sensory input to the central nervous system from the legs. After head removal all the lateral nerve trunks of the prothoracic and mesothoracic ganglia were cut. This removed all sensory input from the legs of these segments. The cuticle above the metathoracic ganglion was then cut and lifted to expose the nerve trunks leaving the ganglion. The trunks, 2, 3 and 4 were cut close to the ganglion on both sides and trunks 5 and 6 on the side not being studied (left) were also cut. The cuticle flap was replaced over the ganglion and a thin layer of petroleum jelly was applied to the edges to prevent drying. The right leg was rotated to expose its dorsal coxal surface and fixed with a fine pin through the medial coxal wall. Nerves 6B and 5r1 were exposed. Nerve branches 5r1a, 5r2, 6A, 6Br2 and 6Br3 were cut. Electrodes were then placed under nerves 6Br4 and 5r1 b and both these nerves were cut distal to the recording electrode. Finally, cutting nerve 5 distal to the branch 5r1 gave a preparation in which all sensory input from the legs was eliminated, and one in which recordings could be made simultaneously from the coxal levator and depressor motoneurones. Strictly this preparation was not de-afferentated because sensory information was probably coming in the abdominal connectives. However, none of this input could be signalling any tactile or proprioceptive information from the legs.

(*c*) *Cut thoracic connectives* (C.T.C.) *preparations.* The connectives between the mesothoracic and metathoracic ganglia were cut, doing minimal damage to the tracheal system. In de-afferentated C.T.C. preparations the meso-metathoracic connectives were cut after de-afferentating the metathoracic ganglion as described in (*b*) above.

3. *Recording and analysing equipment*

All recordings from the nerves were made with single 75 μ silver wire electrodes. The nerve was lifted clear of the haemolymph and coated with petroleum jelly to prevent drying. The two pre-amplifiers used to record simultaneously from nerve 6Br4 and nerve 5r1 (or 5r1 a or 5r1 b) were a Tektronix type 122 and an Isleworth type A101. These amplifiers were connected to a Tektronix 502A oscilloscope and the output from the cathode followers of this oscilloscope was fed to a Thermionic Products T3000 four-channel tape recorder. All experiments were recorded on magnetic tape. This allowed a more detailed analysis of the discharge patterns at a later time. It also allowed the activity to be slowed down so that pen recordings and punched paper tape records could be made of rapid events.

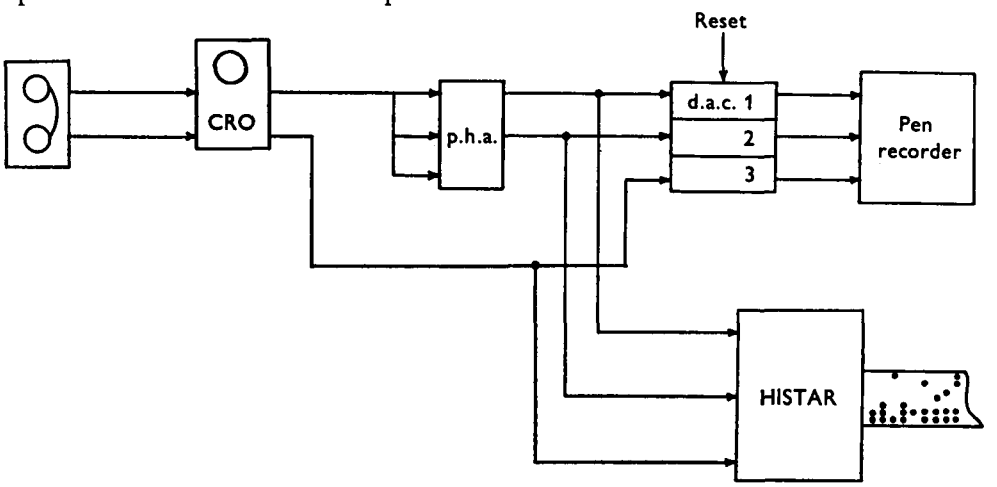

Fig. 2. Equipment layout for analysing activity recorded from nerve 6Br4 and 5r1. p.h.a., pulse height analyser; d.a.c., digital to analog converter; HISTAR, high speed timer and recorder.

The equipment layout for analysing the records made from nerves 6Br4 and 5r1 is shown in Fig. 2. Pulse-height analysing equipment was used to separate the spikes recorded from the different motor axons in nerve 6Br4. Pulse-height analysing was not necessary for separating the spikes from motor axons in nerve 5r1, for generally the largest spike corresponded to activity in the motor axon most intensively studied. Digital-to-analog converters were used to count the number of impulses in a given axon for a unit of time (from 0·1 to 10 sec). The output voltage from these devices was proportional to the number of events occurring in that unit of time (Stein, 1968) and was displayed on a Devices four-channel pen recorder. The result was a plot of average frequency over the unit time interval against time. An on-line pen record of the activity in the different motoneurones was also made during the experiments using this equipment allowing a more rapid selection of interesting activity when analysing.

The activity of up to four motoneurones could be recorded on punched paper tape for subsequent computer analysis, using the device labelled 'HISTAR' in Fig. 2 (Stein, 1965).

4. *Computing procedure*

(*a*) *Bursting activity.* Analysis was performed on the Oxford University KDF9 digital computer. Using the paper tape output of 'HISTAR' bursts of activity in any two specified axons were selected according to the criteria: (1) all intervals were less than 50 msec., and (2) each burst consisted of at least four impulses. Bursts of two motoneurones were regarded as reciprocal when a burst in the second commenced during the cycle time of adjacent bursts in the first (see Fig. 3). When this condition was satisfied for four or more consecutive cycles of activity the parameters defined in Fig. 3 were calculated. The results were accumulated in histograms which agreed with those obtained by measuring photographs but achieved greater accuracy and objectivity (intervals were timed to 0·5 msec.).

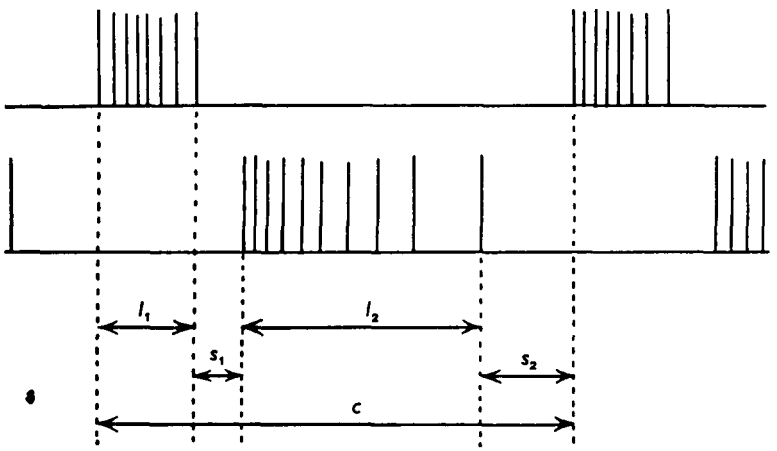

Fig. 3. Terminology for the description of reciprocal discharge patterns. l_1, l_2, burst durations; s_1, s_2, interburst intervals; c, cycle time.

The average frequency variations within bursts were calculated as probability density distributions between the first and last impulse. Bursts within ±10% of specified durations were normalized to the same length and averaged.

(*b*) *Phase and latency correlations.* The phase relationships between events on any two channels of 'HISTAR' output were calculated for intervals of less than 250 msec. Latency histograms between activity in two axons were constructed by a BIOMAC 1000 averaging computer, using pulse-height analysis to discriminate individual units.

(*c*) *Antidromic stimulation.* The BIOMAC computer was used to construct post-stimulus time histograms of the activity in any one axon following single or tetanic stimuli to the same or other axons.

RESULTS

1. *Behavioural observations*

As the general aim of this work was to investigate the neuronal mechanisms underlying rhythmic leg movements it was desirable to study a preparation in which these movements readily occurred either spontaneously or as a result of stimulation of some peripheral receptors. The metathoracic legs of intact animals pinned on

their backs periodically showed spontaneous rhythmic movements but these usually did not last more than a few seconds. In headless animals, however, these spontaneous movements occurred more often and sometimes lasted longer than 10 sec. Stimulation of a cercal hair by a single mechanical shock in quiescent headless preparations usually elicited strong rhythmic movements of the ipsilateral metathoracic leg. Sometimes this stimulus also elicited movements of the contralateral leg, but this usually was neither as prolonged nor as rapid as the ipsilateral response. Cercal stimulation in intact preparations also produced rhythmic leg movements but, as with the spontaneous movements, these generally did not last for more than a few seconds.

Of particular interest in this investigation were the alternate flexion and extension movements at the coxa-trochanter-femur joint. For normal walking Hughes (1952) has shown that there is extension at this joint during leg retraction (depression) and flexion during leg protraction (levation). The muscles that can produce flexion at this joint are the anterior coxal levator (180) innervated by axons in nerve 3b, and the main and posterior coxal levators (181, 182) innervated by axons in nerve 6Br4. The muscles that can produce extension at the trochanter joint are the main depressor muscles (177 A, B, C) innervated by axons in nerve 4, and the coxal depressor muscles (177 D, E, 178, 179) innervated by axons in nerve 5r1. To determine which of these sets of muscles were mainly responsible for producing the alternate flexion and extension movements of the metathoracic femur in an intact animal pinned on its back, either nerve 6Br4 or nerve 4 to one leg was cut and the movements of that leg were compared with the movements of the other metathoracic leg. (Cutting either nerve 6Br4 or nerve 4 does not remove any important sensory input to the central nervous system as Pipa & Cook (1959) have shown that both these nerves are almost entirely motor; sensory fibres from the legs are contained in nerves 3b and 5.) Cutting nerve 4 did not abolish, or have any obvious effect upon, the extension movements at the trochanter joint. Thus the branches A, B and C of the main depressor muscle 177 do not appear to be involved in producing the femoral extension movements. Cutting nerve 6Br4 considerably reduced flexion movements of the femur, and in all preparations the strong flexion characteristic of walking did not occur. Thus the anterior coxal levator muscle, 180, does not produce the strong femur flexion seen during rhythmic leg movements.

The conclusions from these lesion experiments were that the rhythmic movements of the femur relative to the coxa are produced by activity in the motor axons contained in nerve 6Br4 to the main and posterior coxal levator muscles (181, 182) giving flexion movements, and nerve 5r1 to the coxal depressor muscles (177 D, E, 178, 179) giving extension movements. From these observations it was therefore decided to examine the discharge patterns of the motor axons contained in nerves 6Br4 and 5r1 to determine which of these axons were responsible for producing the alternate flexion and extension movements at the trochanter joint. Initially these discharge patterns were studied in headless preparations as the rhythmic leg movements occurred more often and could easily be elicited by cercal stimulation.

2. *Identification of motor axons*

(*a*) *Levator motor axons.* Of the twelve motor axons contained in nerve 6Br4 the six smallest (axons 1–6) could be reliably identified by the amplitude of the extracellularly recorded spikes and their discharge patterns. In intact preparations the axons larger than axon 6 fired in bursts and interaction of the spikes from individual axons made it impossible to identify confidently any of these larger axons. When recordings were made using a single electrode on a section of uncut nerve lifted into petroleum jelly, the other electrode being in the haemolymph close to the restricted length of nerve, the mean peak-to-peak amplitudes of the triphasic spikes recorded from axons 1 to 6 were 0·073, 0·13, 1·2, 1·3, 4·1 and 9·7 mV respectively. The variation in these amplitudes between preparations was very small, the coefficient

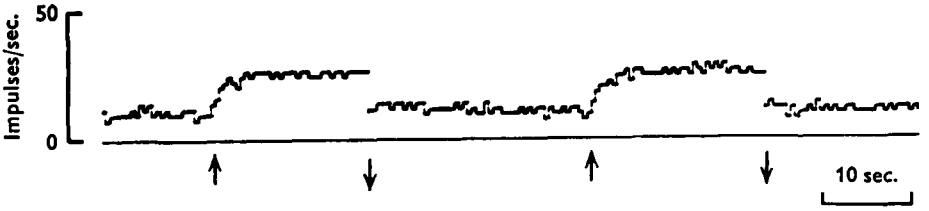

Fig. 4. Effects of femur flexion on the spontaneous discharge rate of the second largest axon in nerve 5r1. The onset of flexion is indicated by the upward going arrows and release from flexion by the downward going arrows.

of variation being about 0·1. The spike amplitudes from axons 3 and 4 were very similar and these axons could not always be identified by spike amplitude alone. However, they could be distinguished when recordings were also made from nerve 4r2a or 5r1, for axon 3 is one branch of a common inhibitory neurone other branches of which are contained in nerves 4r2a and 5r1. Further criteria for identification of axons 5 and 6 were that both axons fired in bursts usually lasting less than 0·5 sec., and that axon 6 was never active without axon 5 being active. Usually axon 6 became active when the discharge frequency of axon 5 exceeded about 80 impulses/sec.

(*b*) *Depressor motor axons.* Recordings from the cut end of nerve 5r1 showed that up to four different motor axons could be spontaneously active at different times. In an intact preparation the spontaneous discharge rate of the largest of these four axons (peak monophasic amplitude of about 6 mV) was usually between 5 and 30 impulses/sec. This resting discharge rate corresponds to that observed by Pringle (1940) for the slow depressor motor axon to the coxal depressor muscles. Further evidence that this motoneurone was in fact the slow depressor comes from the observation that flexion of the coxa-trochanter-femur joint caused an increase in firing rate of this neurone (Fig. 4), and bursts of activity in this neurone corresponded to extension movements of the femur. Also in the resting undissected animal, when this neurone was presumably active, there were no visible signs of twitch contraction in any of the coxal depressor muscles. This slow depressor motor axon will be referred to as axon D_s.

The amplitudes of the monophasic potentials recorded from the three small axons

were all about equal (approximately 0·8 mV). When simultaneous recordings were taken from nerves 5r1 and 6Br4 it was found that one of these three small axons was a branch of the common inhibitory neurone as shown in Fig. 5. The records shown in this figure were taken during a small burst of activity in axon 5 and it is seen that all three small axons became more active during this burst of activity. Generally it was observed that the discharge rate of these axons only increased when the excitatory axons 5 and 6 to the levator muscles became active. Thus the discharge patterns of these neurones suggested they could be inhibitory in function. Intracellular recordings from single fibres of muscle 177E have confirmed that all three small axons are inhibitory (Iles & Pearson, 1969).

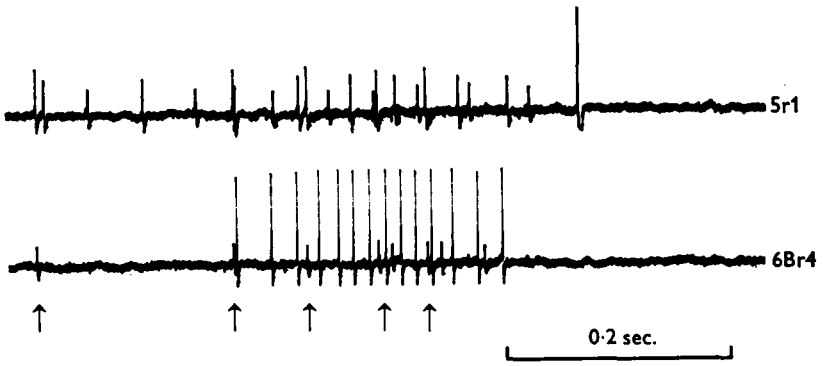

Fig. 5. Discharge pattern of the three smaller axons in nerve 5r1 during a small burst of activity in motoneurone 5. Top trace, extracellular record from nerve 5r1; bottom trace, extracellular record from nerve 6Br4. In the record from nerve 6 Br4 the large spike is from axon 5 and the two smaller spikes from axons 3 and 4 (the spikes from axon 3 are marked with arrows). Note that one of the smaller axons in nerve 5r1 is a branch of the common inhibitory neurone, signified by the 1:1 correspondence of its discharge with that of axon 3. Note also that all three small axons in nerve 5r1 increased in activity during the burst in axon 5. The large spike in the top record is from the slow depressor axon D_s.

When the ipsilateral cercus was lightly touched in the intact animal, the response recorded from nerve 5r1 was often similar to that shown in Fig. 6. Apart from axon D_s being activated, a single large axon (monophasic amplitude of about 25 mV) could also become active. This large axon, when active, gave twitch contractions and corresponds to the fast axon described by Pringle (1939). The large axon will be referred to as axon D_f. The response in axon D_f rapidly declined on repeated stimulation of the cercus, whereas the response in axon D_s, although decreasing, never habituated completely. In some preparations axon D_f could not be activated.

One consistent finding was that on cercal stimulation axon D_s was always activated before axon D_f, and the interval between the first impulse in axon D_s and the first impulse in axon D_f varied from 10 to 150 msec. If axon D_f is responsible for initiating the escape response then this finding may be of relevance in explaining the behavioural observation that the interval between cercal stimulation and the initiation of the escape response is quite variable, ranging from 28 to 90 msec. (Roeder, 1959). Another interesting observation was that a single stimulus to the cercus often gave rise to repeated bursts of activity in axons D_s and D_f, as shown in Fig. 6. Removal of the head, or cutting the thoracic connectives, reduced the response in axon D_f to cercal stimulation. Thus there appears to be some sort of descending

excitatory pathway to control transmission from interneurones in the abdominal cord to axon D_f. Roeder (1948) also reported that transmission through the metathoracic ganglion is reduced by cutting any of the anterior connectives, and behaviourally it is observed that the animal's escape response is reduced (Hughes, 1965*a*).

Nerve 5r1 is short and branches soon after it leaves the main trunk of nerve 5. Recordings from either nerve 5r1a or 5r1b showed potentials corresponding to all the five axons described above. Thus all five axons branch at the first branch point of nerve 5r1. Estimates of axon diameter from monophasic amplitude measurements from nerve 5r1b (method described by Pearson, Stein & Malhotra, 1969) gave the

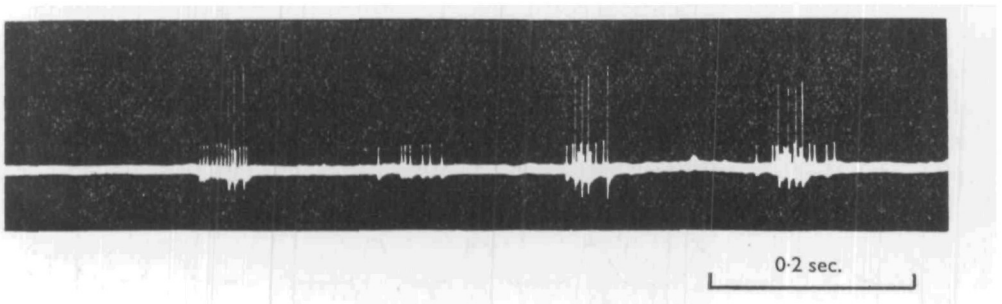

Fig. 6. Response of the slow and fast excitatory axons D_s and D_f in nerve 5r1 to cercal stimulation. The ipsilateral cercus was touched just before the burst of activity on the left. The small spike is from motor axon D_s and the larger from D_f.

diameters of the five axons as 28 μ for axon D_f, 13 μ for axon D_s, and approximately 5 μ for the three smaller axons. In the mesothoracic segment Dresden & Nijenhuis (1958) reported that nerve 5r1 contains five axons larger than 5 μ all of which branched and ran in nerve 5r1a and 5r1b. The diameters of these five axons measured by these workers were one larger than 20 μ, one in the 10 to 20 μ range, and three in the 5–10 μ range. The good correlations of the number, diameter and initial branching of the motor axons contained in nerve 5r1 of the mesothoracic and metathoracic segments suggests that the functional properties of these motor axons may be the same in both segments. Dresden & Nijenhuis (1958) reported that the largest axon (corresponding to axon D_f) innervated only the posterior and anterior coxal depressor muscles (136 and 137) and that the four smaller axons innervated only the two coxal branches of the main depressor muscle (135D and 135E). Usherwood (1962) confirmed that the largest axon innervated only muscles 136 and 137, and showed that this axon gave twitch contractions. He also showed that the other four axons innervated only muscles 135D and 135E and could be classified as two fast and two slow axons. The largest of these four axons (corresponding to axon D_s) was a slow axon. Thus axons D_s and D_f have functional equivalents in the mesothoracic segment. On the other hand the functional properties of the three smaller axons do not appear to be the same since all three of these axons in the metathoracic segment are inhibitory (Iles & Pearson, 1969). However, the discharge characteristics of the three smaller axons in the two segments are identical and, furthermore, at least one of these axons in the mesothoracic segment is a branch of a common inhibitory neurone, this neurone having similar distribution to that described by Pearson & Bergman (1969) for the

metathoracic segment. These observations therefore do not support Usherwood's classification of the three small axons as one slow and two fast.

3. *Motoneuronal activity*

(*a*) *Reciprocal patterning.* The activity of the motor axons contained in nerve 6Br4 could be monitored during rhythmic movements of the femur without damage to any of the coxal muscles. This was achieved by recording from the proximal part of nerve 6B which was exposed by removing the soft cuticle between the dorsal coxal rim and the abdomen. During rhythmic leg movements high-intensity bursts of activity in motor axons 5 and 6 corresponded to the flexion movements about the trochanter joint. Motor axons larger than 6 became active during the more vigorous flexion movements and at least four of these larger axons have been confidently identified in a single experiment. (Interaction of spikes during high-intensity bursts, and the similarity of spike amplitudes from the large axons, meant that it could not be determined whether all six larger axons ever became active during vigorous leg-flexion

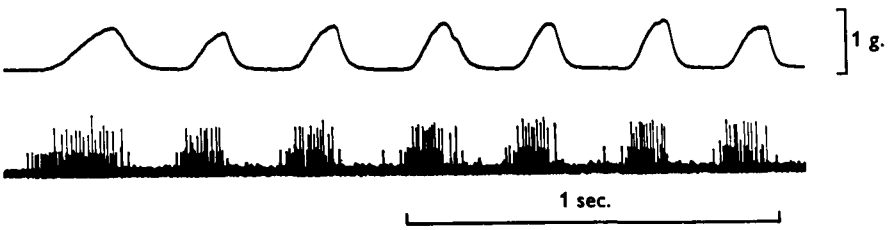

Fig. 7. Strong graded contractions produced in the posterior coxal levator muscles 182C and 182D by bursts of activity in motoneurones 5 and 6. Top trace, tension record; bottom trace, extracellular record from nerve 6B. The smaller spike is from motor axon 5 and the larger from axon 6.

movements.) Since strong flexion movements of the femur occurred when only motor axons 5 and 6 became active, and since cutting nerve 6Br4 abolished these movements, it was concluded that activity in motoneurones 5 and 6 is largely responsible for producing the femoral flexion movements. Bursts of activity in these two motoneurones produced strong graded contractions in branches C and D of the posterior coxal levator muscle, 182, as shown in Fig. 7. At least three of the six axons larger than axon 6 produce twitch contractions in the main coxal levator muscle (181), and the posterior coxal levator muscle is also innervated by one fast axon (Pearson & Bergman, 1969). Twitch responses seen in the tension records from the coxal levator muscles indicated that some of these large fast axons were recruited to produce the very rapid and strong femoral flexion movements.

The activity in the slow depressor motoneurone D_s was strongly reciprocal with the bursts of activity in the levator motoneurones, as shown in Fig. 8. The reciprocal relationship was strongest between motoneurone D_s and the levator motoneurone 5. Only rarely were both simultaneously active and then only when each was discharging at low frequencies (a few impulses per second). To record from nerve 5r1b meant damaging at least muscle 178 and usually muscle 177D. Even so the rhythmic movements of the femur about the trochanter still occurred but, because of the damage to the extensor muscles, the extension movements were not as strong. The activity of

motoneurone D_s could easily be monitored without damage to any of these muscles by recording extracellularly from the coxal depressor muscles. Recordings were made simultaneously from nerve 6B and extracellularly from the coxal depressor muscles during normal rhythmic movements of the femur. No consistent differences have been found in the patterns of activity in the levator motoneurone D_s using these recording conditions compared to those seen when recordings were made from nerves 6Br4 and 5r1 b. Therefore, although some of the coxal depressor muscles were damaged in exposing nerve 5r1 b, leading to an alteration in the movements about the trochanter, this did not significantly alter the discharge patterns in motoneurone D_s and

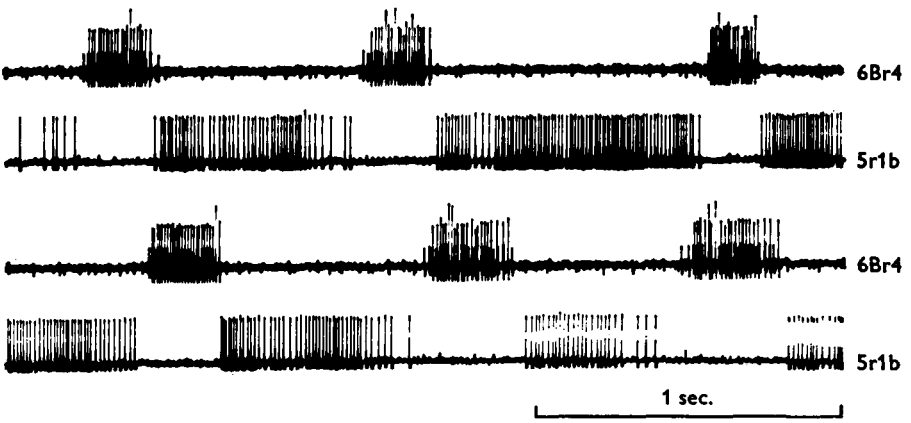

Fig. 8. Reciprocal patterns of activity recorded from nerves 6Br4 and 5r1 b in a headless preparation. The bottom pair of records is continuous with the top. The single spike recorded from nerve 5r1 b is from motor axon D_s. Motor axons 5 and 6 discharged throughout the levator bursts (the spike from motor axon 5 being the smaller in the record from nerve 6Br4). At least one levator motor axon larger than axon 6 discharged one or two times during the levator bursts.

the levator motoneurones. This observation suggested that sensory input from receptors at the trochanter was not of importance in the generation of the reciprocal patterns of activity. A further indication that sensory input was unimportant was that the activity did not depend on whether the leg was completely free to move, or fixed in one position. This was not conclusive, however, for with the leg fixed in one position the possibility that the isometric muscular contractions gave phasic excitation of various groups of leg receptors (e.g. campaniform sensilla), and that this sensory input was important in the generation of the reciprocal patterns of activity, could not be excluded. For this reason recordings were made in the de-afferentated preparation.

The patterns of activity in these de-afferentated preparations were similar in all respects to those seen in the headless animal. Figure 9 shows spontaneous bursts of reciprocal activity seen in one of these de-afferentated preparations. The spontaneous periods of reciprocal activity occurred less often in these preparations but, as with the headless animals, could be readily elicited by stimulation of the cerci.

(*b*) *Characteristics of the reciprocal activity.* As mentioned above, the patterns of activity in the headless and de-afferentated headless preparations were very similar and, apart from the decrease in spontaneous reciprocal activity in de-afferentated

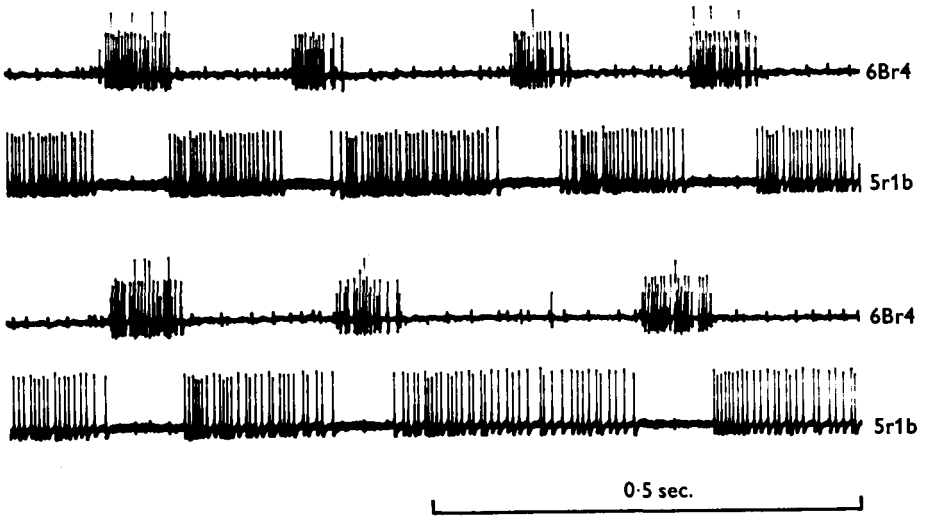

Fig. 9. Reciprocal patterns of activity recorded from nerves 6Br4 and 5r1 b in a de-afferentated headless preparation. The bottom pair of records are continuous with the top. Motor axons 5 and 6 discharged throughout the levator bursts while at least one larger axon became active during some of these bursts (record from nerve 6Br4). Interaction of the spikes from these three axons account for the variability of spike amplitudes in some of the levator bursts. The single spike recorded from nerve 5r1 b is from motor axon D_s.

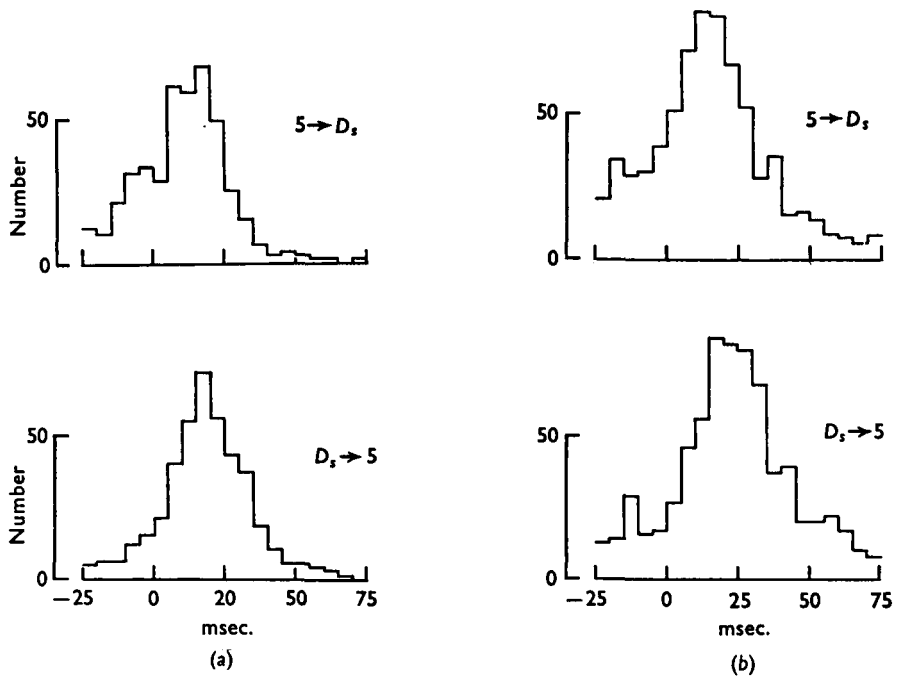

Fig. 10. Histograms of interburst intervals. Top, interval between the last impulse of the burst in motoneurone 5 and first impulse in the burst in motoneurone D_s, and vice versa (bottom), for reciprocal patterns of activity in (*a*) three de-afferentated and (*b*) five headless preparations. Negative values of these intervals when the bursts overlap.

preparations, no consistent differences between the reciprocal activity has so far been found. Therefore the characteristics of this activity discussed in this section apply to both preparations.

Non-overlap. One obvious characteristic was that the bursts in the levator motoneurones were generally non-overlapping with activity in motoneurone D_s. The histograms in Fig. 10 show the interval between the last impulse in motoneurone 5 and the first impulse in motoneurone D_s (top), and vice versa (bottom), observed in three de-afferentated (Fig. 10*a*) and five headless preparations (Fig. 10*b*). These histograms show two commonly observed features: first, there was less overlap between

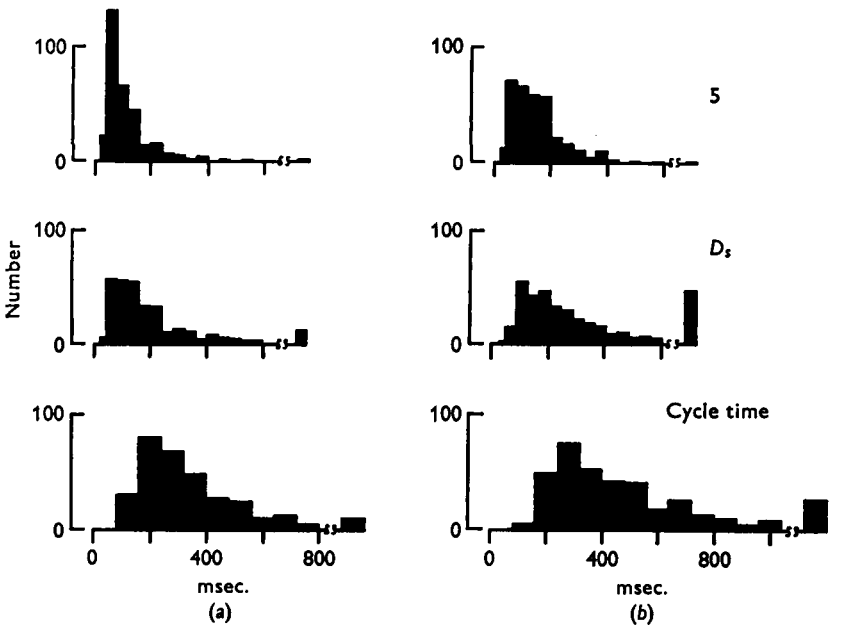

Fig. 11. Histograms of the burst durations in motoneurone 5 (top) and motoneurone D_s (middle), and cycle times (bottom) in (*a*) three de-afferentated headless, and (*b*) five headless preparations. All durations greater than 600 msec. have been placed in the column to the right in each histogram.

the end of activity in motoneurone D_s and the beginning of the burst in motoneurone 5 (see Figs. 8 and 9); and, secondly, the peak of both histograms was always in the range of 10–30 msec., the mean interval between the end of the levator burst and the beginning of the depressor burst being usually smaller than the reverse.

Non-overlapping of reciprocal activity has also been found in insect ventilating systems (Mill & Hughes, 1966; Miller, personal communication) and the locust flight system (Wilson, 1961). The finding that reciprocal patterns of activity are non-overlapping excludes the possibility that activity in inhibitory collateral feedback pathways between the two sets of motoneurones is responsible for terminating the bursts, for considerable overlap in activity would be expected if this were the case. Non-overlapping patterns of reciprocal activity do not indicate, however, that mutual inhibitory feedback pathways between the two sets of motoneurones do not exist; Wilson (1966*b*) has shown that reciprocal non-overlapping activity may be generated

by two groups of negatively coupled motoneurones if positive coupling exists between synergistic motoneurones such that the bursts are initiated by activation of the positive feedback pathways and terminated by fatigue in these pathways. In the cockroach leg system, however, no coupling between synergistic motoneurones has yet been found and often reciprocal patterns could be generated with only motoneurones 5 and D_s becoming active. Therefore, although it cannot be definitely excluded, Wilson's model does not seem to be appropriate for this system.

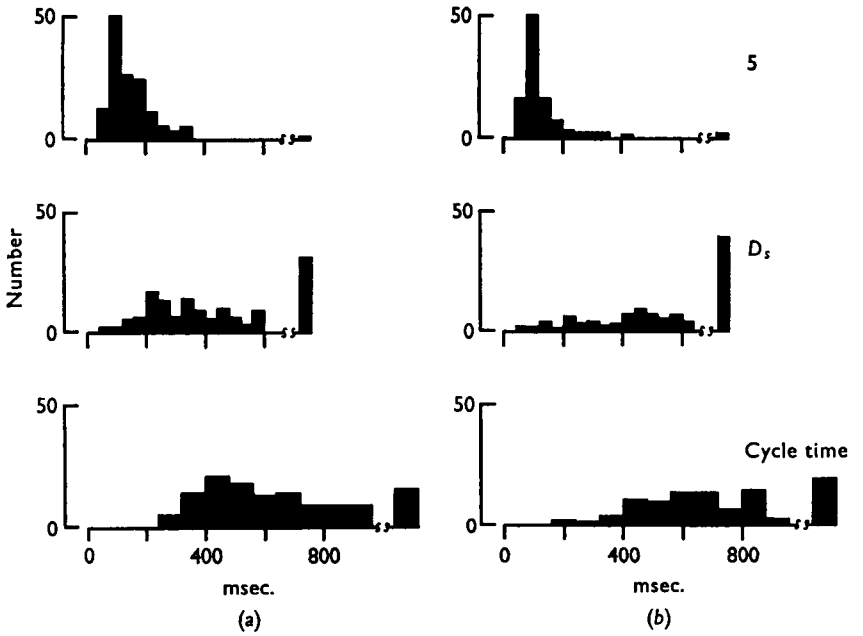

Fig. 12. Histograms of the burst durations in motoneurone 5 (top) and motoneurone D_s (middle), and cycle times (bottom) in (*b*) one cut thoracic connectives and (*a*) three de-afferentated C.T.C. preparations. All durations greater than 600 msec. have been placed in the columns to the right.

Burst durations. Another characteristic of the reciprocal patterns of activity was that the burst durations of the levators were relatively constant compared to those in the depressor motoneurone D_s. The histograms of Fig. 11 show the burst durations of motoneurone 5 and motoneurone D_s for both headless and de-afferentated headless preparations, together with histograms of cycle times for this activity. Only those bursts were counted that were immediately preceded and followed by activity in the antagonistic motoneurone (see METHODS) and included in a period when more than three cycles occurred. The burst durations of motoneurone 5 were usually in the range of 20 to 300 msec. with the peak of the histogram between 100 and 150 msec., the longer bursts tending to be the first in a sequence of reciprocal activity. By contrast the durations of the bursts in motoneurone D_s were sometimes longer than 2 sec. and tended to be more broadly distributed. The peak in the histograms for the burst duration in motoneurone D_s shown in Fig. 11 was a result of a predominance of short cycle times. In preparations with cut thoracic connectives the cycle times were generally more broadly distributed as shown in Fig. 12. This figure also shows histo-

grams of burst duration in motoneurones 5 and D_s observed in one C.T.C. and three de-afferentated C.T.C. preparations. These histograms clearly show the relative constancy of the burst durations in motoneurone 5 compared to those in motoneurone D_s. The plots in Fig. 13 show the relationship between the burst durations

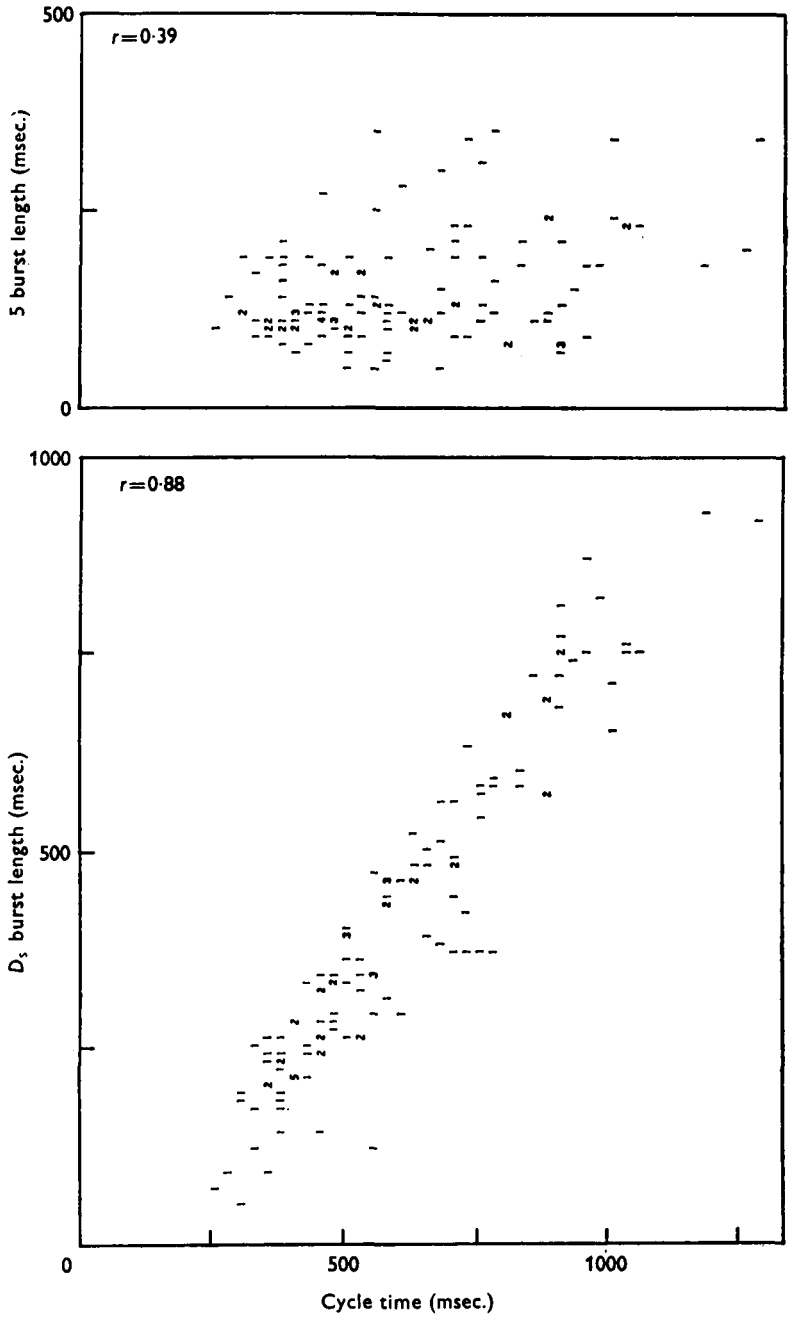

Fig. 13. Scatter diagrams of the burst durations in motoneurone 5 (top) and motoneurone D_s (bottom) against cycle time for three de-afferentated cut thoracic connectives preparations.

in motoneurones 5 and D_s and cycle time for the three de-afferentated C.T.C. preparations. These diagrams show more distinctly that longer cycle times are strongly correlated with long bursts in motoneurone D_s whereas, although there is a slight tendency for the levator burst lengths to increase with cycle time, these remain fairly constant.

In all preparations the burst durations in the levator motoneurone 6 were always shorter than those in motoneurone 5 while the larger levator motoneurones, if active during the levator bursts, discharged only a few times.

Since activity in motoneurones 5 and 6 gives rise to tonic contractions it would be expected that the duration of the mechanical effect produced by the levator bursts would be shorter than the duration of the activity in motoneurone 5. That this is so can be seen from Fig. 7. The finding that the levator burst durations were relatively constant compared with depressor burst durations corresponds to the behavioural observations of relatively constant leg protraction time during normal walking. Recently Delcomyn (personal communication) has shown that during walking the leg protraction time in *Periplaneta* varies from 20 msec. when running to 160 msec. when slowly walking. Therefore the observed durations of the levator bursts correspond reasonably well with these behavioural observations and suggest that the leg protraction phase of walking is predominantly under central control.

Burst shapes. During reciprocal activity the average frequency during a burst of motoneurone 5 varied from 30 to 150 impulses/sec. The discharge frequency of motoneurone 5 remained fairly constant throughout the burst, but showed a slight acceleration and deceleration at the beginning and end of the burst respectively. The pattern of activity in motoneurone 6 was very similar to that of motoneurone 5. Motoneurone 6 only became active when the discharge frequency of motoneurone 5 increased above about 80 impulses/sec. One interesting feature of the levator bursts. was that they did not appear to be dependent upon the activity in motoneurone D_s. Figure 15 shows two very similar levator bursts in one preparation with the activity in motoneurone D_s being very weak before and after one of the bursts and very strong before and after the other. This figure also shows that when the intensity of the bursts was such that motoneurone 6 only fired once, the frequency of discharge in motoneurone 5 was about 80 impulses/sec.

The frequency distribution throughout the bursts of activity in the depressor motoneurone D_s was quite distinctive and differed considerably from that seen in the levator motoneurones 5 and 6. Figure 14 shows the average shape of 16 bursts of activity in motoneurone D_s all of which had durations within the range 360 to 440 msec. The obvious feature of this activity was that the maximum frequency occurred at the beginning of the burst. This decreased to a plateau for long bursts followed by a deceleration at the end of the burst. The peak frequency varied from 100 to 180 impulses/sec. while the plateau was in the range of 60 to 140 impulses/sec. The duration of the initial peak was about 150 msec. and independent of burst length. For shorter bursts the plateau did not occur. One possible explanation for the initial high-frequency activity in motoneurone D_s is that it is produced by post-inhibitory rebound, similar to the rebound effect described by Chalazonitis & Arvanitaki (1961) in motoneurones of the abdominal ganglion of *Aplysia*.

Smaller axons. Apart from the levator motoneurones 5 and 6 and the depressor

motoneurone D_s, the activity in the smaller motoneurones was studied during periods of reciprocal activity to obtain an idea of their normal function. It was not possible to determine the discharge patterns of the levator motoneurones 1 and 2 during periods of reciprocal activity because the small spikes recorded from their axons were obliterated during high-intensity activity in the larger motor axons. Therefore, since the muscles these motoneurones innervate are also not known, nothing can be said about their function.

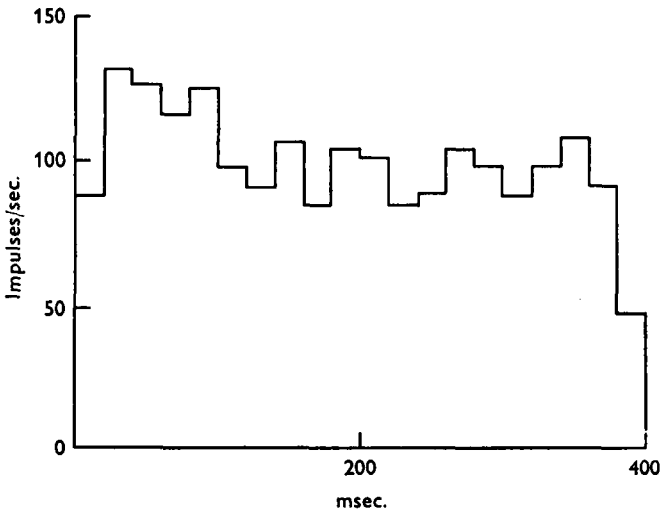

Fig. 14. Average frequency of 16 bursts of activity in motoneurone D_s all of duration in the range 360 to 440 msec. Note that the maximum frequency occurs at the beginning of the burst.

The activity of the common inhibitory neurone and the levator motoneurone 4 depended upon the activity in the depressor motoneurone D_s. When the levator bursts were preceded and followed by high-frequency activity in motoneurone D_s, motoneurone 4 became active near the end of the levator burst but only discharged a small number of impulses. However, motoneurone 4 became considerably more active throughout the levator bursts if the preceding and following activity in motoneurone D_s was low. This is shown in Fig. 15 where two similar levator bursts are preceded and followed by quite different depressor activity. When the depressor activity was high (Fig. 15*b*) motoneurone 4 discharged only once near the end of the levator burst, whereas it became more active during the levator burst when the activity in motoneurone D_s was low (Fig. 15*a*). During walking the activity in motoneurone D_s is high (unpublished observations). therefore the activity in motoneurone 4 is very unlikely to contribute to the production of femoral flexion movements.

Figure 15 also shows that the activity in the common inhibitory neurone depends upon the intensity of activity in motoneurone D_s. The discharge frequency of the common inhibitory neurone always increased during the levator bursts but this increase was most pronounced when the levator bursts were preceded and followed by high-frequency activity in motoneurone D_s. Another characteristic of the discharge of the common inhibitory neurone was that when the activity in motoneurone D_s was intense there was a marked inhibition in the activity of the common inhibitory neurone

after the levator bursts; this is shown in Fig. 15*b*. For weaker activity in the motoneurone D_s this inhibition was not as marked (Fig. 15*a*).

The maximal activity in the other two smaller depressor motoneurones (the third small depressor motor axon is a branch of the common inhibitory neurone) occurred during the levator bursts as shown in Figs. 4 and 15. The intensity of activity in these two motoneurones increased with increasing intensity of the levator bursts.

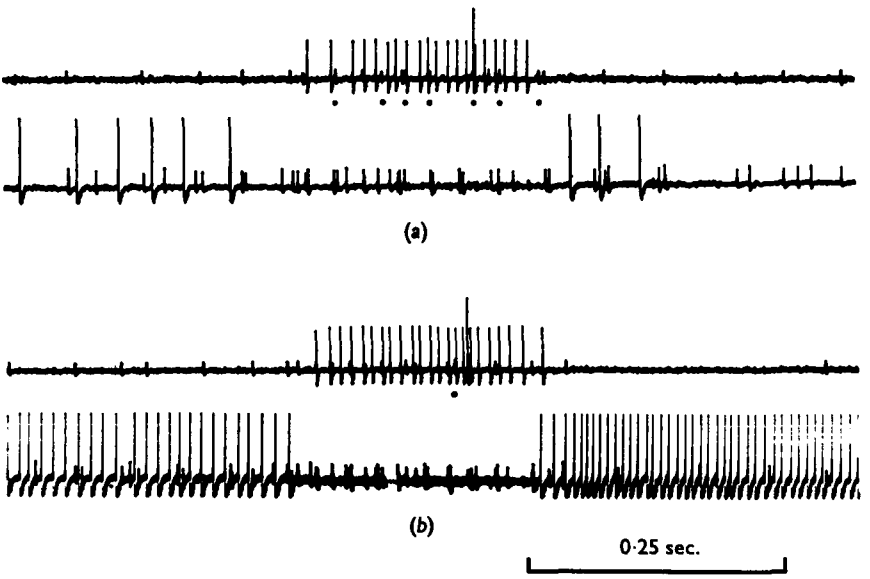

Fig. 15. Activity in levator motoneurone 4 and the common inhibitory neurone during two similar bursts of activity in the levator motoneurones 5 and 6 but different levels of activity in the depressor motoneurone D_s. Top traces, records from nerve 6Br4; bottom traces, record from nerve 5r1 b. The two small spikes in the records from nerve 6Br4 are from axons 3 and 4. These could be distinguished because the firing of axon 3 showed a 1 : 1 correspondence to the firing of another axon in nerve 5r1 (axon 3 is one branch of a common inhibitory neurone another branch of which is contained in nerve 5r1 b). The spikes from axon 4 have been marked with dots. The two larger spikes recorded from nerve 6Br4 are from axons 5 and 6, axon 6 discharging only once during each burst. The large spike in the record from nerve 5r1 b is from axon D_s. The three small axons in nerve 5r1 b discharge maximally during the levator bursts. Note also that when the activity in motoneurone D_s is high, (*b*), the activity in motoneurone 4 is low, while the common inhibitory neurone discharges at a higher rate throughout the levator bursts and is inhibited at the end of this burst.

(*c*) *Non-reciprocal activity*. One type of non-reciprocal activity was that the levator motoneurones 5 and 6 would discharge in bursts without motoneurone D_s becoming active, as shown in Fig. 16. The reverse situation, i.e. motoneurone D_s firing in normal bursts without motoneurones 5 and 6 becoming active, has never been seen. The generation of levator bursts without activity in motoneurone D_s is similar to the finding in the locust flight system that depressor bursts may be generated without levator activity (Waldron, 1967). Also in the insect ventilation system expiratory bursts can occur without any activity in the inspiratory motoneurones (Miller, personal communication). Often levator bursts were immediately followed by a small number of impulses in motoneurone D_s, but the depressor activity was not maintained over the entire interval between the levator bursts (Fig. 17). These patterns of activity

support the proposal of a rebound effect on motoneurone D_s giving rise to the increase in excitation after a levator burst (cf. Fig. 8 in Chalazonitis & Arvanitaki, 1961). Again the reverse pattern has never been seen. Thus three patterns of burst activity have been observed: bursts in only the levator motoneurones, bursts in the levator motoneurones followed by a short burst of activity in motoneurone D_s, and fully reciprocal activity.

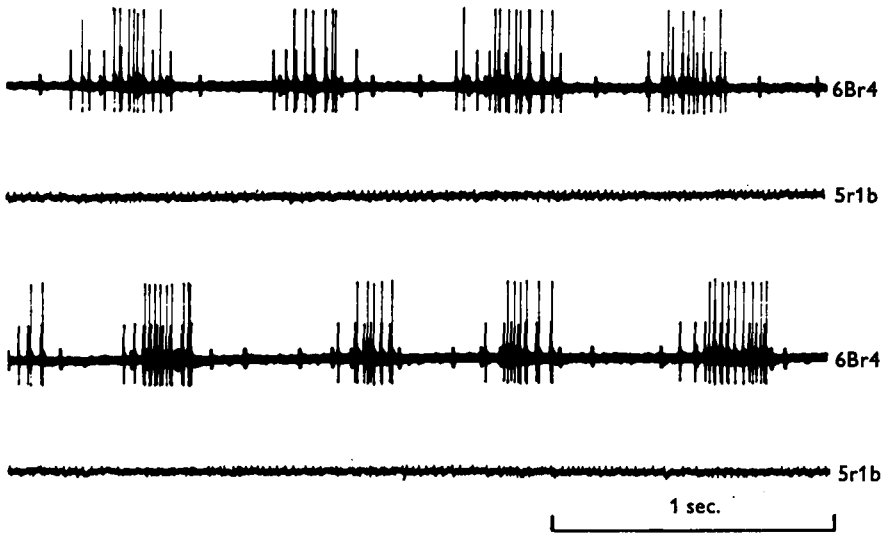

Fig. 16. Bursts of activity in levator motoneurones 5 and 6 without activity in motoneurone D_s. The bottom pair of records is continuous with the top. The two largest spikes in the record from nerve 6Br4 are from axons 5 and 6, and the small spike from axon 3. The reciprocal activity shown in Fig. 9 was from the same preparation.

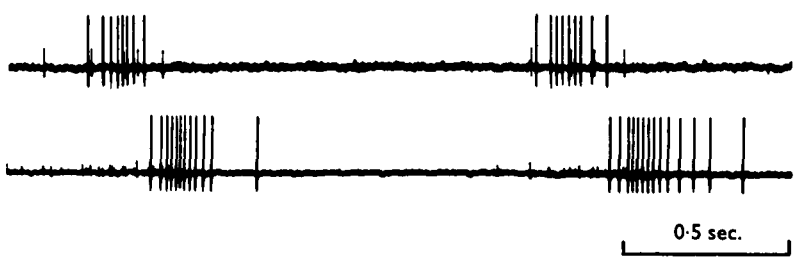

Fig. 17. Bursts of activity in levator motoneurone 5 (large spike in top record) followed immediately by short bursts of activity in motoneurone D_s (lower record).

Another type of non-reciprocal activity was that motoneurones 5 and D_s would be simultaneously active for long periods. This occurred very rarely and then only when each motoneurone was discharging at low frequencies (a few impulses/sec.). Slow fluctuations in the average frequency of the two motoneurones were negatively correlated; an increase in the frequency of motoneurone 5 corresponded with a decrease in frequency of motoneurone D_s, and vice versa. During these periods no latency or phase correlations have been found between the two trains of impulses as might have been expected if there was any direct coupling between the motoneurones.

(*d*) *Antidromic stimulation.* Another test for coupling at the motoneuronal level was to stimulate antidromically either the levator motor axons or the depressor motor axons and observe the effect of this stimulation on the activity in the other.

Nerve 6Br4 was sufficiently long to allow both stimulating and recording electrodes to be placed on the nerve (the stimulating electrode being the distal pair). When the stimulus was adjusted so that all axons larger than axon 2 were excited, a single stimulus re-set the activity in all the larger motoneurones. This re-setting effect on the activity of motor axons 3, 4 and 5 is shown in Fig. 18. The interval immediately after the stimulus was significantly longer than the mean interval before the stimulus, which was probably due to an accumulation of refractoriness (Wilson, 1964). Miller (1967) has also found the same effect on antidromically stimulating the motor axons innervating locust spiracle muscles.

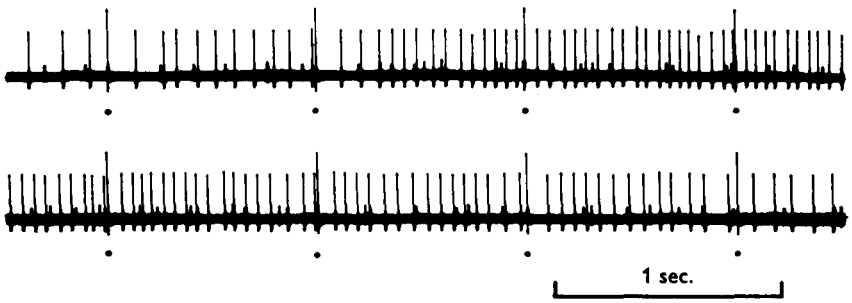

Fig. 18. Re-setting effect of single antidromic stimuli on the activity in the motor axons 3, 4 and 5 of nerve 6Br4. Stimuli were delivered at 1/sec. (dots) distal to the recording electrode. The two small spikes are from axons 3 and 4 (the spike from axon 3 being slightly larger) while the large spike is from axon 5.

High-frequency (> 50/sec.) antidromic stimulation of nerve 6Br4 led to a depression of all spontaneous activity in motor axons contained in that nerve which recovered with a time constant of about 0·5 sec. Kennedy, Evoy & Fields (1966) report a similar finding for the motor axons innervating the flexor muscles of the crayfish abdomen. These depression effects only occurred when the axon under study was stimulated, thus, if the inhibition was mediated via collateral feedback pathways (similar to Renshaw inhibition in the mammalian spinal cord), then these collaterals only produce inhibition in the motoneurones from which they originate. Rather than collateral feedback pathways mediating the depression effects a simpler possibility is that the high-frequency antidromic stimulation leads to some form of inactivation of the spike-generating region which slowly recovers at the end of stimulation.

High-frequency antidromic stimulation of the motor axons larger than axon 2 in nerve 6Br4 was without effect on the activity of motoneurone D_s. (Another control for stimulus strength was that the branch of the common inhibitory neurone in nerve 5r1 b was activated by each stimulus.) Similarly antidromic stimulation of the axons in nerve 5r1 b was without effect on the activity in the levator motoneurones (it was assumed that if the branch of the common inhibitory neurone in nerve 6Br4 was activated then motor axons D_s and D_f, which are larger than the branch of the common inhibitory neurone in nerve 5r1 b, were antidromically activated).

These negative results exclude the existence of collateral pathways that can be activated antidromically. The re-setting effect did show that the spike-initiating zone was invaded. Therefore, if collaterals do exist, they must have a separate spike-initiating zone.

DISCUSSION

1. *Central patterning of motoneuronal activity*

The patterns of activity seen in the de-afferentated headless preparations were in all respects similar to those recorded in the headless preparations. The reciprocal patterning of motoneuronal activity is therefore largely independent of sensory feedback from leg receptors. Wilson (1966, 1967) has postulated the existence of central neuronal oscillators in each half-ganglion responsible for patterning activity in antagonistic motoneurones. The findings presented in this paper provide strong evidence for the existence of such oscillators in the metathoracic ganglion. Similar oscillators also exist in the mesothoracic ganglion, and the ipsilateral oscillators in the mesothoracic and metathoracic ganglia are centrally coupled in such a way that there is a negative correlation between activity in the two sets of levator motoneurones (Iles & Pearson, in preparation).

Central patterning of motoneuronal activity has been demonstrated in many other arthropod systems where rhythmic movements occur, e.g. flight in locust and fly (Wilson, 1961, 1968; Wyman, 1965, 1966), ventilation in locust dragonfly and cockroach (Miller, 1965; Mill & Hughes, 1966; Farley, Case & Roeder, 1967), and movements of swimmerets in crayfish and lobster (Hughes & Wiersma, 1960; Davis, 1968, 1969). One feature of the insect ventilation and crustacean swimmeret systems is that the duration of movement in one direction is relatively constant for varying cycle times (the inspiratory phase of insect ventilation and the power stroke of swimmeret movements). This is also a feature of insect walking where the leg-protraction time is relatively constant compared to leg-retraction time. (Hughes, 1965*b*, has reported that for the cockroach, *Periplaneta americana*, the ratio of leg-protraction time to retraction time varies from 0·07 for very slow walking to 1 for rapid running.) The finding that the durations of centrally generated levator bursts are relatively constant compared to the durations of depressor bursts parallels this behavioural observation (during leg-protraction there is flexion of the femur as a result of contractions in the coxal levator muscles) and is similar to the findings that the centrally generated bursts of activity in locust inspiratory motoneurones and the lobster swimmeret motoneurones remain fairly constant for varying cycle times (Miller, 1965; Davis, 1969).

There are two aspects of the centrally generated motoneuronal activity that require explanations in terms of the connectivity and properties of cells within the central nervous system; the first is the generation of the levator burst, and the second is the strong reciprocal relationship between activity in the levator motoneurones, particularly motoneurone 5, and activity in motoneurone D_s. There are a number of different but not mutually exclusive methods by which levator bursts could be generated. The simplest is that burst activity is an intrinsic property of the motoneurones themselves. If this is the mechanism for levator-burst formation then inhibitory collateral feedback pathways must exist from motoneurone 5 to motoneurone D_s in order to account for the strong reciprocal relationship between the bursts of activity in these neurones.

Since no evidence for the existence of such pathways has been suggested by anti dromic stimulation experiments, by the non-overlapping of reciprocal activity, or by the absence of phase and latency correlation between the spike trains from motoneurones 5 and D_s when simultaneously active, the possibility that levator bursts are due to the intrinsic properties of the motoneurones seems unlikely. The same difficulty arises with the proposal that the levator bursts are generated as a result of positive coupling between the synergistic motoneurones, analogous to that proposed by Wilson (1968) for the locust flight system. Another point against this second possibility for burst generation is that levator bursts could often be generated with only motoneurone 5 becoming active. Moreover, when the larger levator motoneurones were also active there were no signs of coupling of activity in different motor axons similar to that for coupling of flight motoneurones in the locust where strong latency correlations have been found between activity in synergistic motoneurones (Wilson, 1968).

Rather than levator bursts being generated at the motoneuronal level a more likely possibility is that their generation results from phasic driving of the levator motoneurones by a single (or set of) bursting interneurone(s). Davis & Murphy (1969) have made a somewhat similar suggestion for the lobster swimmeret system by postulating that motoneurones are driven by a sinusoidal input, this input arising from activity in driver interneurones. This mechanism is known to be largely responsible for the burst generation in motoneurones of the lobster cardiac ganglion (Hagiwara & Bullock, 1957) but in this system there is also coupling between motoneurones (Watanabe, 1958) and positive feedback from motoneurones to the pacemaker interneurones (Watanabe & Bullock, 1960). One characteristic of the levator bursts to be explained is their relative constancy in duration. Little can be said on this problem at the moment as no direct evidence from intracellular records is available and nothing can be inferred from the discharge patterns. One possibility is that bursting driver interneurones have properties similar to those described by Chalazonitis (1963) for the bursting interneurone in the abdominal ganglion of *Aplysia* where the interburst interval in this neurone could be decreased or increased by passing depolarizing or hyperpolarizing current respectively but the burst durations remained fairly constant.

Often levator bursts were generated without motoneurone D_s becoming active (Fig. 16) or with motoneurone D_s discharging a small burst of impulses at the end of the levator burst (Fig. 17). The reverse patterns have never been seen which suggests that the bursts of activity in motoneurone D_s are not produced by independently bursting driver interneurones. The maximum rate of firing in motoneurone D_s occurred at the beginning of its bursts (Fig. 14) and as mentioned earlier, this could have been due to a post-inhibitory rebound effect. If inhibitory collateral feedback pathways between the antagonistic sets of motoneurones do not exist then the simplest model for explaining the bursts of activity in motoneurone D_s is that the bursting driver interneurones to the levator motoneurones also inhibit the ongoing activity in motoneurone D_s, as shown schematically in Fig. 19. In this model it is proposed that a post-inhibitory rebound in the activity of motoneurone D_s follows its release from inhibition at the end of the burst in the levator driver interneurones. By comparison with some other invertebrate systems this proposal seems reasonable for post-inhibitory rebound excitation has been clearly demonstrated by Chalazonitis

& Arvanitaki (1961) in motoneurones of *Aplysia*, while the termination of hyperpolarizing currents leads to rebound excitation of the giant cells in the leech abdominal ganglion (Eckert, 1963) and in some of the follower cells in the lobster cardiac ganglion (Hagiwara & Bullock, 1957). The independent bias on motoneurone D_s shown in Fig. 19 accounts for the observation that for very similar levator bursts the depressor motoneurone D_s can be almost completely inactive or firing reciprocally at very high frequencies (Fig. 15). For a certain range of low levels of bias on motoneurone D_s this neurone will not fire continuously between levator bursts but will be excited reboundwise on release from inhibition after a burst of activity in the levator driver interneurone. Therefore this model also accounts for the type of activity where a burst in motoneurone 5 is immediately followed by a small burst of activity in motoneurone D_s.

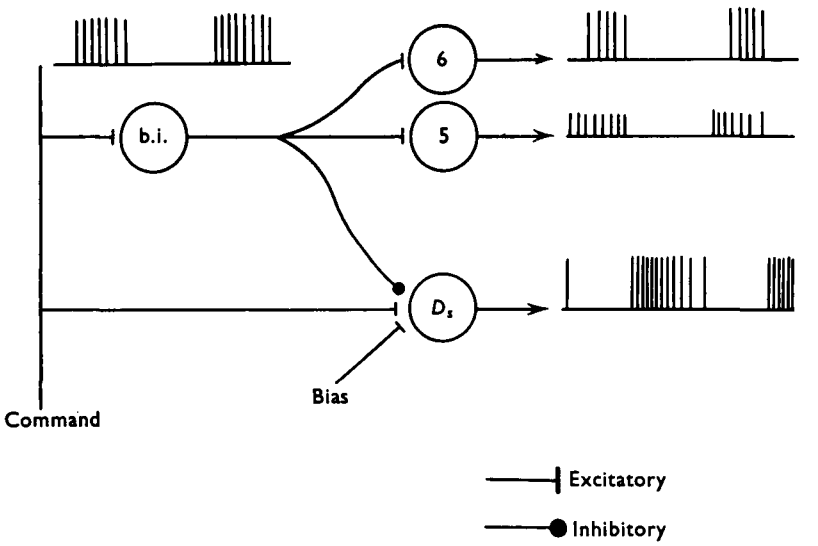

Fig. 19. Hypothetical model for the generation of reciprocal patterns of activity. Levator motoneurones 5 and 6 are phasically driven by a bursting interneurone, b.i., which also inhibits activity in depressor motoneurone D_s. The bias on motoneurone D_s accounts for its varying levels of activity for similar levator bursts. See text for further details.

The intensity of the burst in motoneurone D_s generally increased with decreasing cycle time (unpublished observation), and strong reciprocal patterns of activity are usually initiated without any of the motoneurones 5, 6 or D_s previously being active. To account for both these findings it is proposed that activity in command interneurones (shown in Fig. 19 as the common input to the bursting interneurone, b.i., and motoneurone D_s) leads to burst generation in the initially quiescent interneurones driving the levator motoneurones, and that an increase in the level of the command input decreases the interburst intervals and increases the intensity of activity in motoneurone D_s. One unpublished observation of relevance to this proposal is that regular stimulation of filaments of the ipsilateral meso-metathoracic connective has sometimes led to generation of reciprocal patterns of activity which only persisted so long as the filament was being stimulated, and the cycle time decreased with increasing stimulus frequencies.

The above model is the simplest to account for our experimental results to date. With the lack of other evidence it is pointless to speculate about other more complex models. Only by directly recording from cells within the ganglion, together with histological studies, will the cellular events and cell connectivity leading to the patterning of motoneuronal activity in this and other arthropod systems be determined (Bentley, 1969*a*, *b*). The results presented in this paper show that the centrally generated patterns of activity in the coxal levator and depressor motoneurones have many similarities with those seen in other systems, which raises the general question as to what extent do common principles of neuronal organization underlie the patterning of motoneuronal activity.

2. *Functional significance of the motoneuronal activity*

En passant recordings from nerve 6B during rhythmic leg movements have shown that the activity of motoneurones contained in this nerve are very similar to those observed in the de-afferentated headless preparation (*en passant* recording from nerve 6B allowed the recording of motoneuronal activity without damage to any coxal muscles). Motor axons 5 and 6 were always active throughout flexion movements of the femur while some of the larger axons were often recruited during very rapid movements. Since femur flexion movements were produced when only axons 5 and 6 became active, and since cutting nerve 6Br4 abolished these movements, then the bursts of activity in motoneurones 5 and 6 contribute significantly to the flexion movements of the femur by producing rapid graded contractions in the posterior coxal levator muscle (Fig. 7). The durations of the levator bursts occurring in the de-afferentated headless preparation during periods of reciprocal activity were relatively constant compared to the burst durations in motoneurone D_s. Furthermore, these levator-burst durations correspond to the behaviourally observed leg-protraction times. These findings suggest that the motoneuronal activity producing the leg-protraction phase of walking is almost entirely centrally generated, i.e. generated largely independent of sensory feedback from leg receptors. Although direct recordings have not been made of activity in levator motoneurones during walking behaviour, it is likely that the activity in these cells is similar to that occurring during the rhythmic leg movements of restrained inverted animals, and therefore similar to the centrally generated levator bursts shown in Figs. 8 and 9. This is a reasonable possibility because the sensory input from leg receptors during the protraction phase of walking will be similar to that during flexion movements of the femur and tibia in the inverted animal. Sensory input from the other legs which would not occur in restrained animals could affect levator motoneuronal activity during leg protraction. However, these effects will be small (Pringle, 1940; Wilson, 1965) and unlikely to alter significantly the patterning of the levator motoneuronal activity.

Extracellular recordings from the coxal depressor muscles have shown that during extension movements of the femur in both restrained inverted preparations and in unrestrained freely moving animals, the slow depressor motoneurone D_s discharges at rates of up to 200 impulses/sec (unpublished observations). These bursts were very similar to those seen in the de-afferentated headless preparations. As bursts of activity in motoneurone D_s give rise to strong rapid graded contractions of the coxal depressor muscles, a significant part of the femur extension movements during walking is

probably produced by activity in this motoneurone, and the generation of this activity may, to a large extent, be independent of sensory feedback from leg receptors.

Apart from activity in motoneurones 5, 6 and D_s and the other larger axons, the functional significance of the patterns of activity in the smaller levator and depressor motoneurones must be considered. The muscles innervated by levator motoneurones 1 and 2 have not been determined, nor has it been possible to investigate the discharge patterns in these cells as the small size of their spikes prevented study of their patterns of activity when the larger motor axons were active. Thus nothing can be said about their function. The common inhibitory neurone discharged maximally during the levator bursts and was inhibited at the beginning of the depressor bursts (Fig. 15*b*). Stimulation of the common inhibitory neurone produces inhibition of contractions produced by motoneurone D_s in the coxal depressor muscles (unpublished observations) and may therefore function to produce a more rapid relaxation of the depressor muscles after a burst of activity in motoneurone D_s as suggested by Pearson & Bergman (1969). The relaxation time-constant in the coxal depressor muscles 135D and 135E of the mesothoracic segment after high-frequency stimulation of the axon equivalent to motor axon D_s is about 300 msec. (Usherwood, 1962). Since the leg protraction time is less than 160 msec. some form of active relaxation of the depressor muscles would therefore seem desirable. The other two small depressor axons are also inhibitory (Iles & Pearson, 1969) and discharge maximally throughout the levator bursts. The increased activity in these two neurones during levator bursts would assist the common inhibitory neurone in producing a faster relaxation of the coxal depressor muscles. Levator motoneurone 4 produces very slow graded contractions in the posterior coxal levator muscles (Pearson & Bergman, 1969) but is not strongly activated during periods of reciprocal activity (Fig. 15*b*). Thus this motoneurone most probably functions to produce slow flexion movements of the femur when the animal is making postural adjustments.

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SUMMARY

1. Observation of movements of the metathoracic legs of the cockroach before and after section of peripheral nerves allowed identification of muscles involved in flexion and extension of the femur.

2. Extracellular recordings from the nerves to these coxal muscles show that during rhythmic leg movements bursts of activity in a number of levator motor axons were strongly reciprocal and generally non-overlapping with those of a slow depressor motor axon.

3. These reciprocal patterns persisted after removal of all sensory input from the legs.

4. The durations of levator bursts were relatively constant compared to those of the depressor, corresponding to the behavioural observations on leg protraction time. The pattern was asymmetric: levator bursts could be generated without depressor activity, but never the reverse.

5. No evidence was found for inhibitory collateral pathways between antagonist motoneurones.

6. It is proposed that levator motoneurones are driven by a group of bursting interneurones which simultaneously inhibit the ongoing depressor activity.

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