THE DERIVATION OF THE MOTOR COMMAND
TO THE SPIRACLES OF THE LOCUST

BY P. L. MILLER

Department of Zoology, Oxford, England

INTRODUCTION

Among insects several patterns of motor activity have been studied in which the sensory input bears no simple relation to the output. Of these most is known of the locust flight system as a result of the penetrating studies of Wilson (1961, 1964) and Wilson & Weis-Fogh (1962). Wilson & Wyman (1965) applied electrical shocks with random timing to the locust central nerve cord and produced patterned oscillations in groups of motoneurones which supply the flight muscles, similar but at lower frequency to those which occur naturally in flight. The ventilatory mechanisms of some insects show similarities with the locust flight system in so far as maintained stimulation, either electrical or by the application of gases, can produce patterned oscillations in neurones which bring about pumping movements (Huber, 1960; Mill & Hughes, 1966; Miller, 1966). Unlike the flight system, however, such activity may also persist in the absence of input. The spiracle motoneurones too show persistent activity in the absence of input so that in the intact insect they need to be periodically switched off by other cells to allow the spiracle to open. In common with many cells in the arthropod cord their 'resting' state is apparently one of continuing activity. They are comparable with the spontaneous units of unknown function which have been studied in the crayfish cord (Preston & Kennedy, 1962; Kennedy & Preston, 1963; Biederman, 1964; Takeda & Kennedy, 1965; Biederman-Thorson, 1966), and some of their properties are also comparable with those of the neurones of the crustacean cardiac ganglion (summary by Hagiwara, 1961).

In the present study an attempt has been made to distinguish between the contributions of a pacemaker, which is an integral part of the motoneurone, and of synaptic input to the motor patterns which emerge under different conditions in the intact insect. Spiracle nerves contain only a small number of axons and it is normally feasible to distinguish their separate activity in extracellular records from the whole nerve. This, together with the fact that functionally related components of the system are distributed in different ganglia, makes the preparation a suitable one for a study of this nature. Moreover, the number of different commands issued is limited since, unlike most motor systems, the spiracle muscles are engaged in only one form of activity, namely, opening or closing the valves. Some attempt at an interpretation of the results in terms of synaptic properties and the organization of the axon branches in the neuropile has been made, but confirmation must await direct measurements from this region. Recent studies of the electrical activity in the neuropile of insects (Iwasaki & Wilson, 1966; Roeder, 1966) suggest that this approach will soon bear fruit.

In normal ventilation the first (prothoracic) spiracle of the locust, *Schistocerca gregaria*, opens for a short period during the inspiratory stroke of the abdomen and is closed for the remainder of the cycle. In co-operation with other spiracles this activity permits the abdominal pump to produce a uni-directional ventilating airstream which passes posteriorly through the insect. The valve movements are brought about by two muscles, an opener and a closer. The closer receives two motor axons from the median dorsal nerve of the prothoracic ganglion (G I), while the opener is supplied by three motor axons; two arise in G I and accompany the nerves to the closer, while the third arises in the mesothoracic ganglion (G II) and proceeds to the opener in II N 1 and then joins the median nerve close to the spiracle (Fig. 1) (Miller, 1960*a*, 1965). In spiracle 2 of the same species, Hoyle (1959) has shown that all muscle fibres are doubly innervated by a 'slow' and a 'fast' axon, whose effects on the muscle differ only by a small amount. Unlike many motor systems both contribute at roughly equal frequencies to normal activity, and their differing slow and fast properties may not be important.

MATERIAL AND METHODS

Schistocerca gregaria Forskål and *Locusta migratoria migratorioides* (Reiche and Fairme), both kindly supplied by the Anti-Locust Research Centre, were used in these studies. Most experiments were performed on teneral adults of *Schistocerca* but mature insects have also been examined. The results are derived from a total of 84 preparations. The temperature was normally 20–22° C.

Records were taken from the transverse nerves to the spiracles by conventional methods which have already been described (Miller, 1960*a*). Electrical stimulation of nerves was carried out through fine platinum wire electrodes via an isolation unit, using square-wave pulses of 0·2 msec. duration at various frequencies and intensities. Stimulation of parts of a connective between G I and G II was carried out after it had been desheathed and split into a number of small bundles, according to the method recommended by Fielden & Hughes (1962). One connective was cut through completely, while the other was usually split into six to twelve bundles. After removal of the tough perineurium there was often a temporary conduction block, probably due to unavoidable stretching of the fibres, but recovery usually followed in less than 1 min. as indicated by the re-establishment of synchrony between spiracle 1 and abdominal pumping. Recordings taken from split bundles showed that the activity of recognizable units persisted for at least 1 hr. when the preparation was bathed in Hoyle's locust saline. Split bundles were lifted into air on the stimulating electrodes and surrounded by a small amount of petroleum jelly to prevent desiccation and minimize stimulus spread. The recording electrodes on the spiracle nerves were similarly treated and then reimmersed in saline (Fig. 1). No deleterious effects were observed as a result of the use of petroleum jelly (cf. Narahashi, 1963).

As Fielden & Hughes (1962) have pointed out, a disadvantage of this technique is that in successive preparations there is no means of identifying the same bundles of fibres except by their activity. A variety of effects is to be expected therefore when different bundles are stimulated electrically, according to their component fibres. On the termination of an experiment the bundle was squeezed between forceps on the ganglion side, subsequent shocks then being found to be without effect on the spiracle

nerves. This indicated that the stimulated area was normally close to the electrodes, although with very high intensities of current it was sometimes possible to stimulate the motoneurones directly. In all preparations care was taken to minimize damage to the tracheae which supply the ganglia.
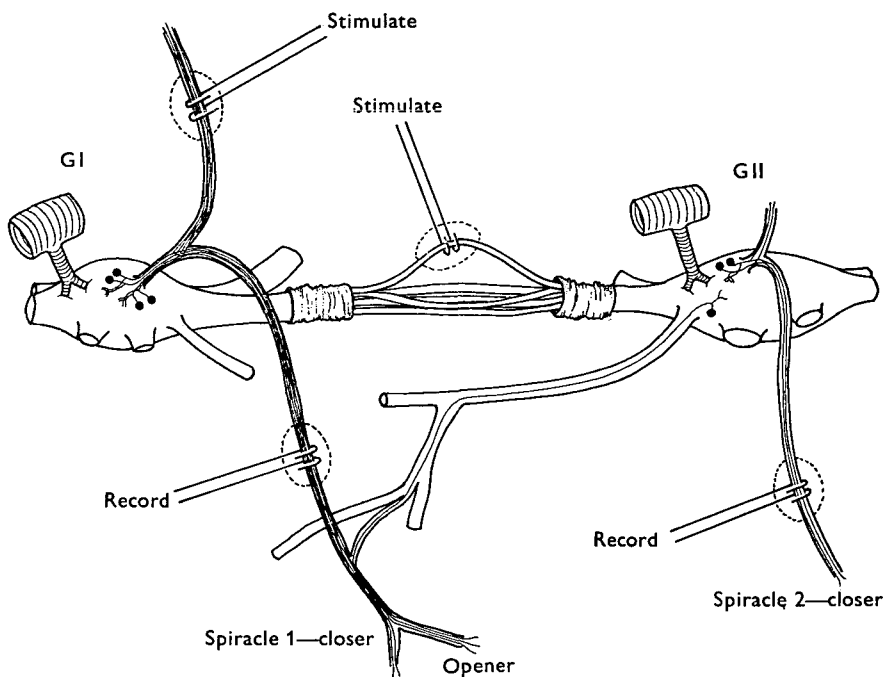

Fig. 1. *Schistocerca*. Diagram to illustrate the stimulating and recording sites on the transverse nerves and split connectives between the prothoracic (G I) and mesothoracic (G II) ganglia. The motor supply to the spiracle muscles is also shown.

RESULTS AND COMMENTS

Observations on the natural patterns of activity. A brief description of the natural patterns of activity has already been given (Miller, 1960*a*, 1965): only activity in the two motor units to the closer muscle of spiracle 1 will be described here although similar patterns are distributed to spiracle 2. In favourable preparations their separate activity can be distinguished by the different spike heights, or one can be examined alone after damage of the axon of the other proximal to the electrodes. Three main patterns of activity can be distinguished (Figs. 2, 3).

Pattern 1 consists of high-frequency bursts of impulses occurring at the same frequency in both axons, between 100 and 250/sec./axon, and lasting about 100 msec. The two units fire either synchronously or alternately and the burst normally initiates contraction of the muscle and closure of the spiracle. The pattern comprises smooth regular firing, sometimes at a slowly declining frequency, and is usually quite distinct from pattern 2 which follows. Such bursts may occur synchronously in spiracles 1 and 2, but can occur in either alone; in the former case there is no synchronization of individual spikes in the two spiracles (Fig. 2 *c, d*).

Pattern 2 normally follows 1 although it may be absent during fast ventilation.

When pumping is slow or stops temporarily, pattern 2 continues in the spiracle nerves and maintains a tetanus in the closer muscle until the next inspiratory stroke starts. Again both axons participate, firing at approximately equal frequencies (20–50 impulses/sec./axon). Pattern 2 may comprise either *a*, regular trains of impulses, often
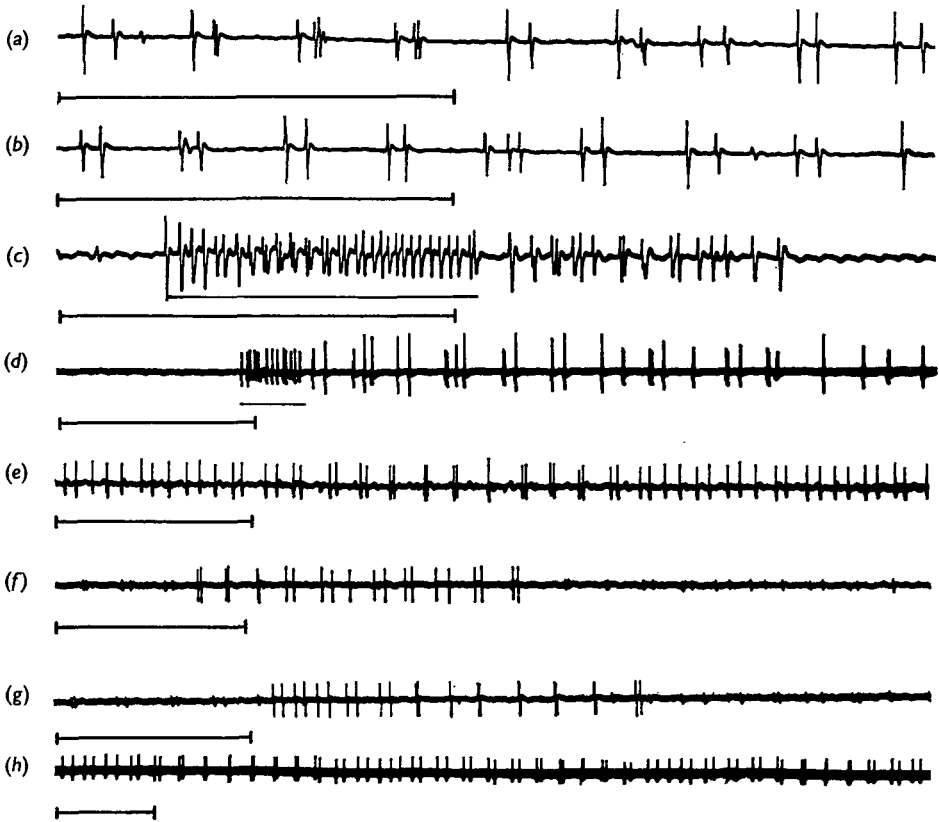

Fig. 2. Patterns of motor activity in the axons to the closer muscle of spiracle 1, in intact locusts (*a–g*), and after the removal of G III (*h*). (*a, b*) Pattern 2*a* in *Schistocerca*: synchronous impulses in each of the two axons (large spikes) occur in couplets. (*c*) A long high-frequency burst (pattern 1), underlined, in *Locusta* followed by pattern 2*b* during fast ventilation. (*d*) A short high-frequency burst, underlined, in *Schistocerca* followed by pattern 2*a*. (*e*) Pattern 2*c* in *Schistocerca* during a pause in ventilation; each unit fires regularly but independently. (*f, g*) Short expiratory trains in *Locusta* in which no high-frequency burst occurs and pattern 2*c* is seen. (*h*) Free running in *Schistocerca* after removal of G III; compare with *e* above. Horizontal scale below each trace = 200 msec.

Legend to Fig. 3

Fig. 3 A. Plot of consecutive impulse intervals in the motor nerve to the closer muscle of spiracle 1 of *Schistocerca* (both axons) during a complete expiratory stroke: pattern 1 is seen followed by 2*c*. Points at O on the vertical scale indicate impulses synchronized in the two axons. B, spike interval histogram for the spiracle 1 motor units showing pattern 2*a* with a limited amount of couplet firing; most impulses are synchronized in the two axons (interval = 0–1·75 msec.) C, spike interval histogram for the spiracle 1 motor units showing pattern 2*a* with strong couplet formation. The three major intervals of 0, 12 and 42 msec are distinct. D, spike interval histogram as in B and C for free-running units in a preparation with G III removed: no impulse synchronization occurs.
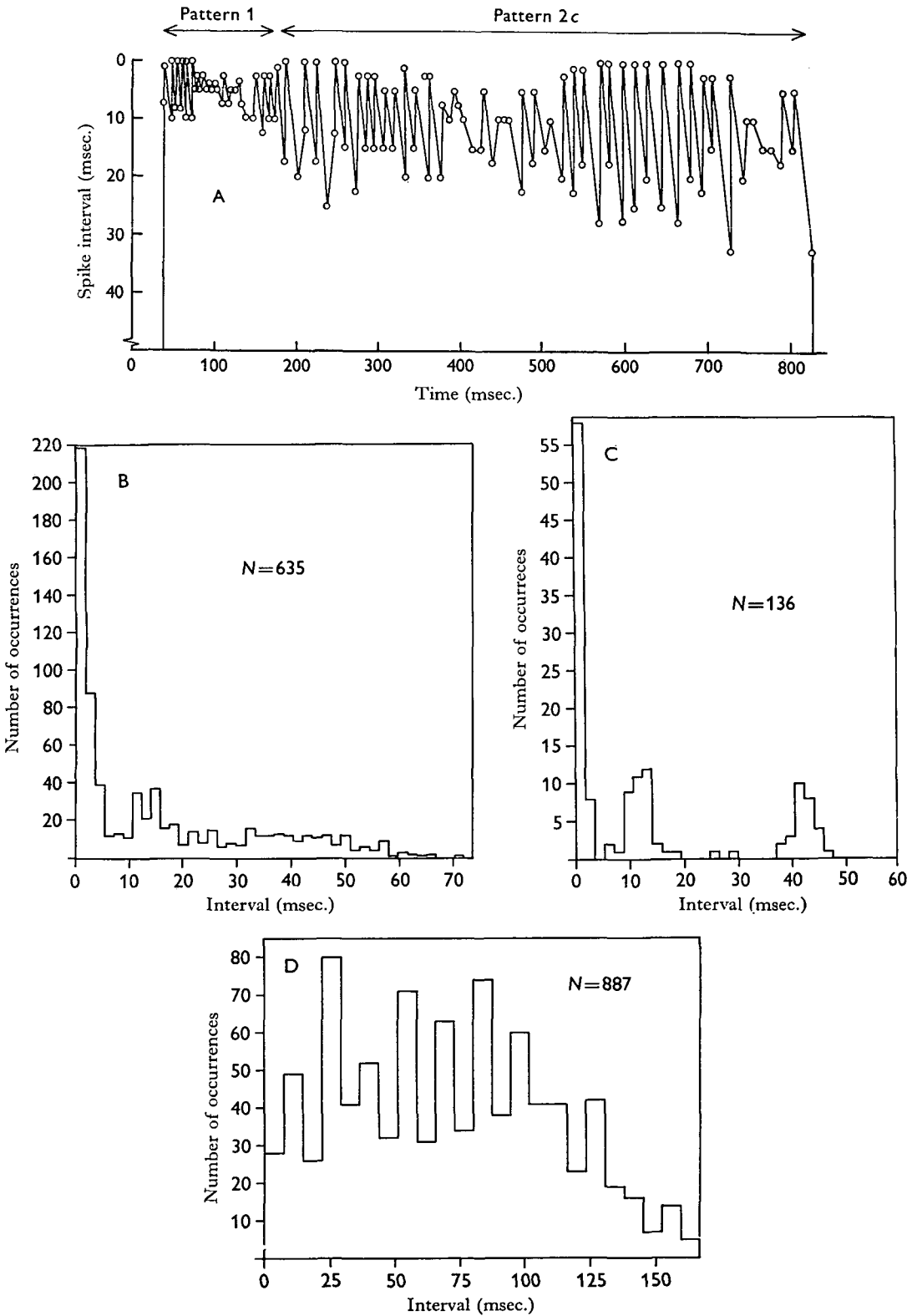
Fig. 3. For legend see opposite page

in doublets (Figs. 2 *a, b*; 3B, C), synchronized in the two units; or *b*, less regular firing in which sometimes components of *a* or *c* can be recognized; or *c*, regular evenly spaced but independent trains of impulses in the two axons, usually at different frequencies so that a slower beat-frequency appears (Figs. 2 *e, f, g*; 3D). Pattern 2*c* is identical with *free running* which always appears after removal of the metathoracic ganglion (GIII),
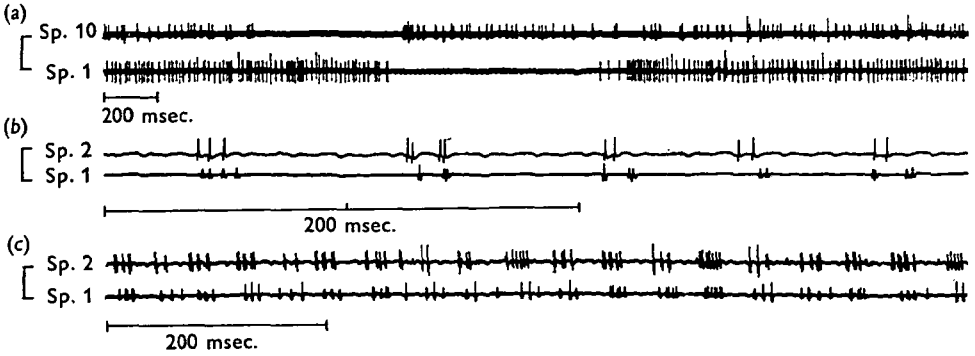

Fig. 4(*a*). Simultaneous records from the motor nerves to spiracles 1 and 10 of *Schistocerca* during one ventilatory cycle. Only the activity in units supplying the closer muscles is seen: in spiracle 10 activity ceases during expiration and in spiracle 1 during inspiration. (*b, c*) Simultaneous records from the motor nerves to spiracles 1 and 2 showing approximate synchronization between impulses during pattern 2*a* firing, spiracle 2 usually leading by about 5 msec.
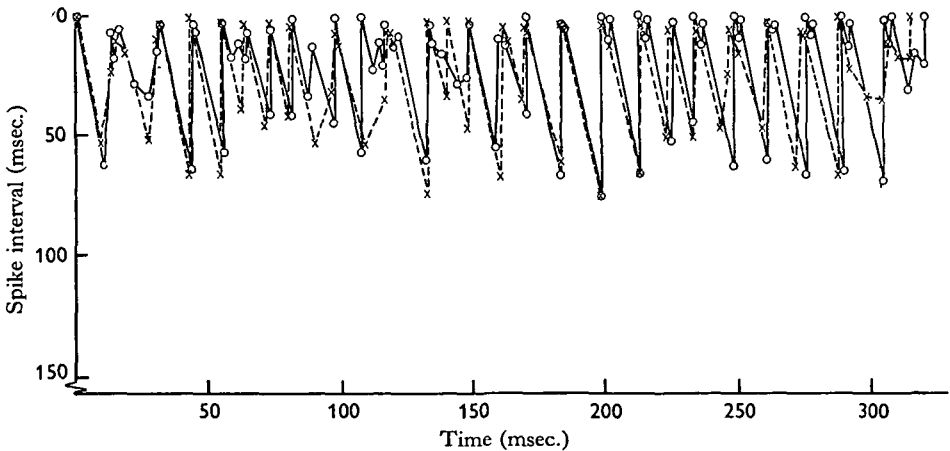

Fig. 5. Plots of consecutive impulse intervals (both axons) during pattern 2*a* firing when impulse synchronization occurs between spiracles 1 (O—O) and 2 (× - - - ×). Points occurring at o in the vertical scale indicate the synchronous firing of both units of a spiracle.

or section of the connectives between GI and GII. Pattern 2*c* is more commonly seen in intact *Locusta* than in *Schistocerca*, although in the latter 2*a* is sometimes replaced by 2*c* at faster frequencies of ventilation. Different individuals, however, tend to remain true to their firing plan; for example, one *Schistocerca* showed pattern 2*a* before and after decapitation, removal of the abdomen and section of lateral thoracic nerves, even though ventilation frequency was altered considerably.

When pattern 2*a* appears there is often approximate 1:1 synchronization of impulses between spiracles 1 and 2 (Figs. 4*b*, *c* and 5). This has not been observed during 2*b* or *c*, or under any conditions after removal of GIII. Since at high frequencies patterns 2*a*, *b* and *c* all produce a maintained tetanus of the closer muscle it may be functionally unimportant which firing plan is followed. However, tension measurements of the muscle contractions have not been made and close-paired impulses such as occur in 2*a* are well known in other situations to cause tensions higher than are produced by the same frequency evenly spaced (Wilson & Davies, 1965); Hoyle (1959) has shown this to be the case in spiracle 2 of the locust.

In summary, therefore, pattern 2 comprises activity which is either largely synchronized (impulse for impulse) in both axons and in adjacent segments, or which is independent in each axon, or again which may be irregular and possibly a mixture of the first two.

Pattern 3 comprises short periods of no activity in the nerves when the muscle relaxes and the spiracle opens coincident with the inspiratory stroke. During weak ventilation, activity in both axons of spiracle 2 may continue at a low frequency, but this does not normally occur in the spiracle 1 nerve. As a result spiracle 2 may remain more or less closed throughout the cycle and inspiration takes place only through spiracle 1, spiracles 3 and 4 also remaining closed. At such times spiracle 10 acts as the only expiratory spiracle and gaseous exchange is achieved with a minimum of spiracle opening. Similarly in resting dragonflies spiracles 1 and 10 alone are operational.

The synchronization of ventilatory activity throughout the insect

In order to produce effective uni-directional ventilation, spiracle movements must be precisely synchronized with the pumping strokes and these strokes must take place in all parts of the abdomen nearly simultaneously. Activity is co-ordinated in the locust by GIII which lies one-tenth of the distance between spiracles 1 and 10. To bring about synchronized movements it must therefore excite remote regions through fast-conducting pathways with minimum delays or operate through a system with graded delays. Co-ordination in the abdominal cord is believed to be brought about by fast-conducting interneurones (Miller, 1966) similar to those which occur in the cricket (Huber, 1960), and a further set has been postulated which runs anteriorly to harness the activity of the thoracic spiracles to the pumping rhythm (Miller, 1965).

Some measurements have been made of the delays between the occurrence of events in the ventilatory cycle in different parts of the insect. For example, bursts of impulses in lateral nerves from GIII to expiratory muscles commence up to 10 msec. before corresponding high-frequency bursts in the spiracle 1 nerve. Comparable bursts leave the last abdominal ganglion in nerves to expiratory muscles about 60 msec. after the start of activity in the spiracle 1 nerve, while a similar delay intervenes between the end of closer activity in the spiracle 1 nerve and the start of the high-frequency burst in the nerve to spiracle 10 (Fig. 4*a*). In dragonfly larvae Mill & Hughes (1966) found a relatively long delay, over 50 msec, between the expiratory bursts in adjacent abdominal segments, and exceptionally a delay of as much as 379 msec. between segments 5 and 7. Ventilation would therefore presumably have more of a peristaltic nature than in locusts.

The onset of high-frequency bursts in the spiracle 2 nerve precedes that of corres-

ponding bursts in the spiracle 1 nerve by 5–7 msec. When impulse synchronization occurs between these spiracles (pattern 2a), spiracle 2 normally leads by about 5 msec., although considerable variation is seen (Figs. 4, 5). Such synchronization does not involve all the spikes; for example, in one preparation a train in the spiracle 1 nerve comprised a total of 246 spikes (both axons) of which 61 % were within 5 msec. of a corresponding spike in the spiracle 2 nerve. The frequency was 25/sec./axon, and since most spikes occurred synchronously in the two axons of spiracle 1 (cf. Fig. 3 B, C), the impulse intervals for the whole nerve were about 0 and 40 msec. Correspondingly in the spiracle 2 nerve, firing at 20/sec./axon, 68 % of the spikes were synchronized with spiracle 1 spikes, and many of the remainder were only a little more than 5 msec. from a spiracle 1 spike. In contrast, when pattern 2c occurs, less than 3 % of the spikes are precisely synchronized in the two axons of spiracle 1 (cf. Fig. 3D) and a similarly small proportion is synchronized between spiracles. Pattern 2c is thus characterized by a lack of intra- and intersegmental coupling while these are prominent in pattern 2a.

Localization of the spiracle motoneurone pacemaker

Free running occurs indefinitely in the motoneurones to the closer muscles of spiracles 1 and 2 after section of the connectives between GII and GIII, and, as in pattern 2c, there is no interaction or coupling between the units. Section of one GI–II connective and the contralateral GII–III connective produces continuous free running in spiracle 1 while spiracle 2 remains synchronized with ventilation; the command interneurones therefore run through GII from GIII to GI. In a preparation in which GI is connected only with the head, stimulation of the head, mechanically or by light, accelerates free running which then slowly returns to its original frequency. Comparable stimulation of GI does not have this effect. If the head becomes hypoxic, the frequency of free running declines and it may cease. Since spiracle 1 supplies the head directly with tracheae, control of its activity by the head is to be expected. After decapitation free running becomes insensitive to mechanical stimulation applied at any point.

Electrical stimulation has been applied to the spiracle nerves antidromically in an attempt to locate the pacemakers responsible for free running. Stimulation was applied to one transverse nerve while records were taken from the contralateral nerve. This method takes advantage of the fact that the median nerve and its contained motor axons divide into left and right transverse nerves before running to the spiracles. Single shocks evoked responses (S spikes) in all four motor axons (two supplying the closer and two the opener) which could be distinguished readily by their different heights and conduction rates (Miller, 1960a), and these were recorded together with natural impulses produced by the pacemaker (P spikes). When S and P spikes collided in the transverse nerve, no S spikes were recorded, but when collision took place in the median nerve no P spikes were recorded. Otherwise it was found that S spikes were always followed by at least the normal spike interval before the next P spikes appeared, regardless of the position of the S spikes in the natural P cycle. Thus both pacemaker cycles could be re-set by antidromic spikes which must therefore be able to invade the pacemaker loci (Figs. 6, 7). It can be concluded that each motoneurone contains an independent pacemaker, perhaps with impulse production and recovery cycle taking place at the same locus, and there is no need to postulate an antecedent pacemaking

cell. The alternative theory of a closed loop of cells which has been discussed by Kennedy & Preston (1963) can be discounted since there is no evidence for the necessary long refractory periods. Kennedy & Preston reached the same conclusion for the cells they were studying in crayfish ganglia. Biederman-Thorson (1966) has shown that many of the spontaneously active cells in crustacean ganglia derive their activity from intrinsic pacemakers, even when their firing pattern is not markedly regular, such cells having 'noisy' pacemakers.
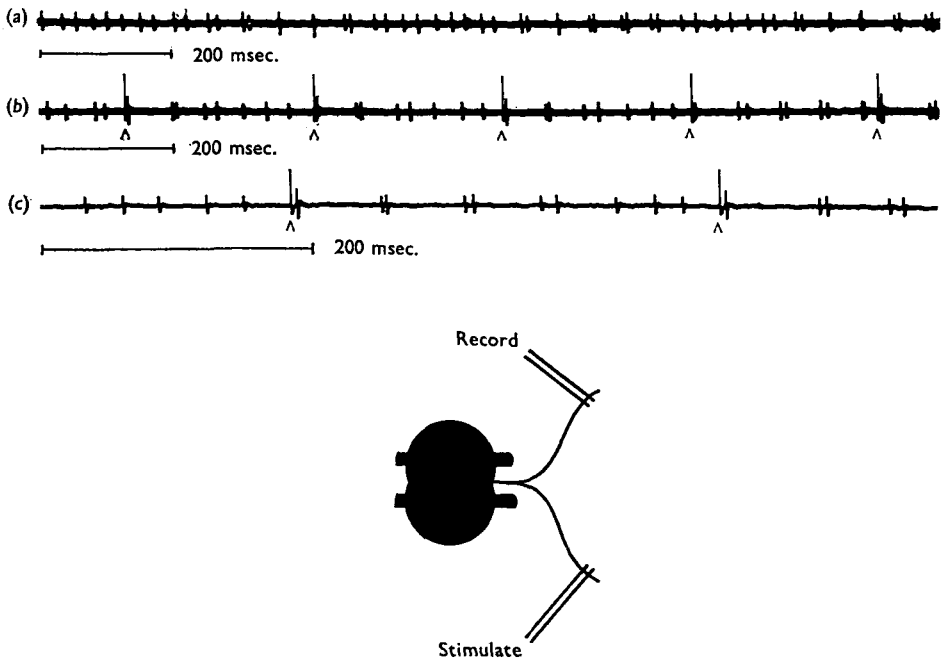

Fig. 6. Antidromic stimulation of and recordings from the transverse nerves of an isolated G I. (*a*) Free running without stimulation. (*b*, *c*) Stimulation with single shocks (∧), which re-phase the spontaneous cycles of each motoneurone.

In some records the interval between the S spike and the subsequent P spike in the spiracle motor nerve was slightly longer than the normal pacemaking interval. This was most marked when the S spike followed closely after the preceding P spike and some examples are shown in Fig. 7. The juxtaposition of P and S spikes may produce a prolonged post-excitatory depression, or the delay may represent the activity of a negative feed-back mechanism which controls pacemaker frequency (cf. Bullock & Horridge, 1965, p. 314). However, the possible effects of stimulus spread cannot be excluded.

Normal pacemaker activity can be studied most readily in preparations in which one axon has been damaged proximal to the electrodes and the activity of the other alone is recorded. A histogram of the activity of one pacemaker recorded in this way is shown in Fig. 8, where it can be seen that the mean interval is 38 msec. with extreme values at 28 and 46 msec.
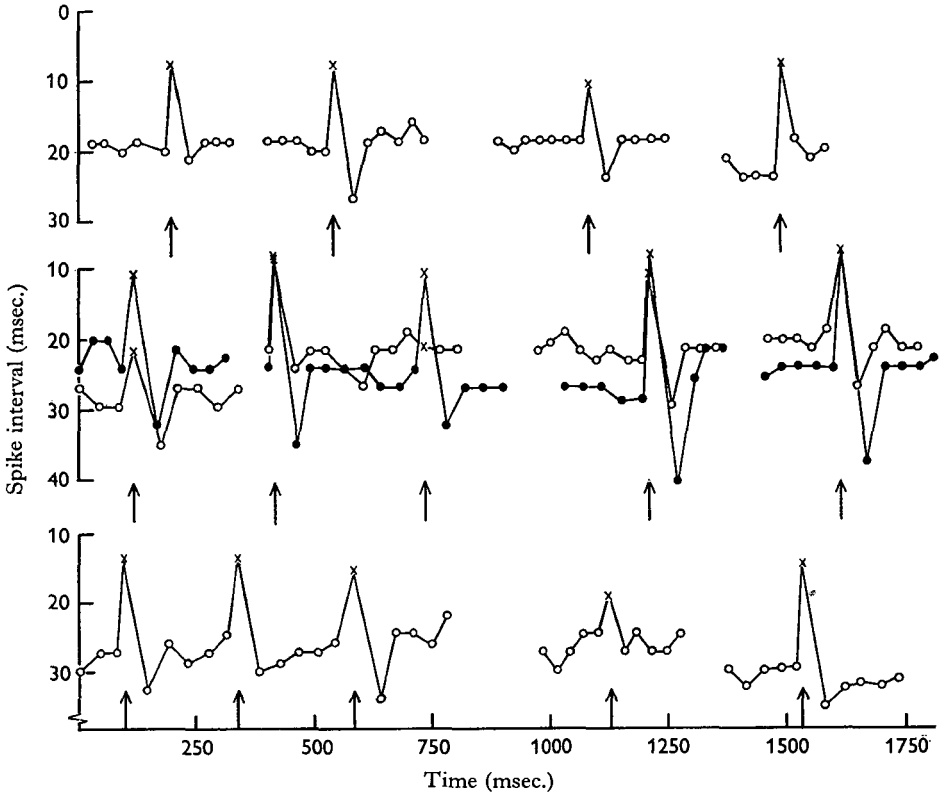
Fig. 7. Separate plots of consecutive impulse intervals for each motor unit supplying the closer muscle of spiracle 1. In the middle line, both units are shown. At arrows, antidromic shocks are given to the transverse nerve as in Fig. 6. ×, stimulated spikes; O, pacemaker spikes. Stimulated spikes are sometimes followed by intervals greater than the normal spike interval.
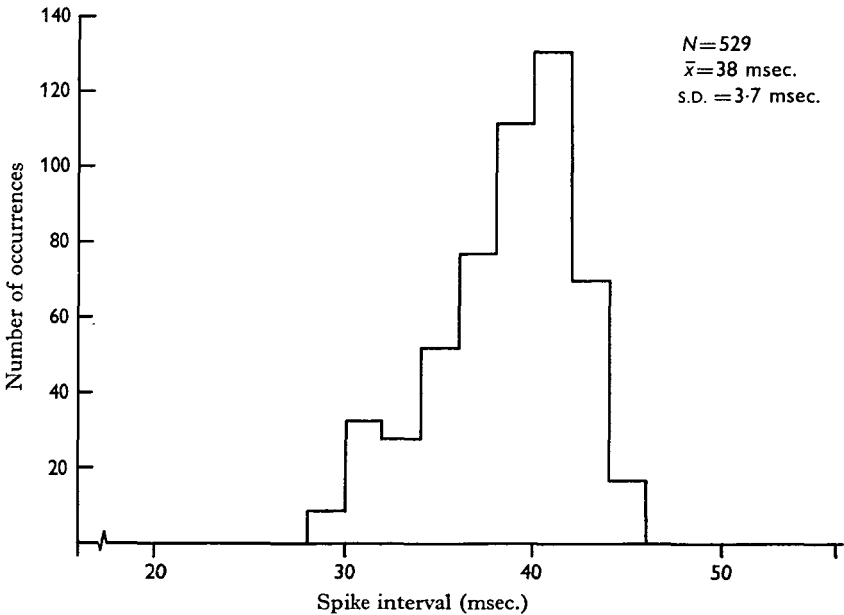

Fig. 8. Spike interval histogram for the activity of one spiracle 1 motor unit of *Schistocerca*. G III has been removed and the unit is free running.

The origin of motor activity in intact preparations

Antidromic stimulation of the transverse nerve has been applied to preparations with an intact nerve cord in an attempt to determine the contribution of the moto-neurone pacemakers to normal activity. When *Locusta* ventilates rapidly pattern 2*b* may be distinguished, but if ventilation is slowed by perfusion with 50 % oxygen, or by decapitation, pattern 2*b* is often replaced by 2*c*; that is, firing becomes more regular. During periods of 2*c* firing, antidromic stimulation re-sets the cycle as in free running. Again the close proximity of P and subsequent S spikes sometimes results in a lengthened interval before the next P spike (Fig. 9*c* and *d*). Pattern 2*c*, therefore, like free running, seems to be solely due to the activity of the pacemakers which act independently of each other and of those in G II.
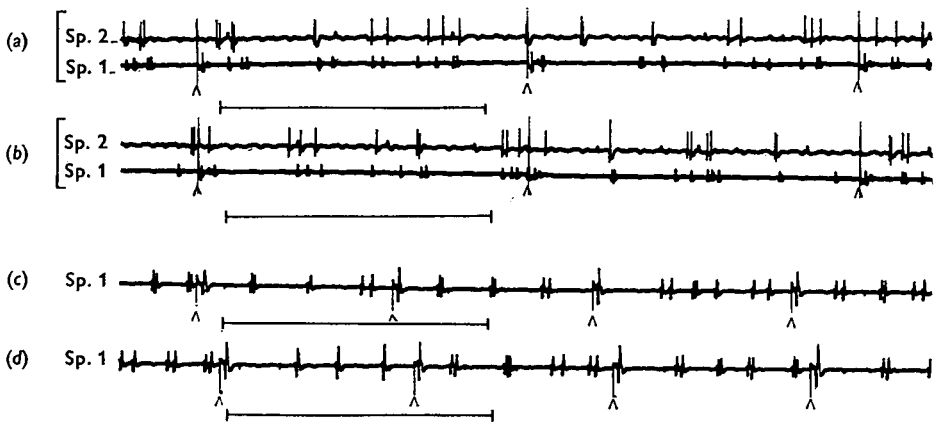

Fig. 9. (*a, b*) Simultaneous records from the transverse nerves to spiracles 1 and 2 of *Schisto-cerca* with cord intact during the delivery of single shocks (∧) to the transverse nerve of spiracle 1 (lower line). Pattern 2*a* appears and antidromic stimulation does not affect the timing of the next natural impulse. (*c, d*). Records from spiracle 1 of *Locusua* during pattern 2*c* firing when antidromic stimulation re-sets the timing of the next natural impulse. Horizontal scales below each trace = 200 msec.

When pattern 2*a* appears, however, antidromic stimulation produces S spikes which are followed by natural spikes after a more variable interval. Moreover simultaneous records from spiracles 1 and 2, stimulation being applied to spiracle 1 alone, show that such natural spikes are often matched by spikes in the spiracle 2 nerve (Fig. 9*a, b*). The spiracle 2 records act as a control for those from spiracle 1 and they suggest that S spikes are interpolated into the firing train without affecting the timing of subsequent spikes. Although spiracle 1 may be firing couplets at regular intervals, the timing at least of the first spike is not affected by the S spikes, and it can be concluded that during such firing the oscillation with the major period is determined by synaptic input and that the pacemakers are probably inactive.

In conclusion, therefore, pattern 2*c*, identical with the free running which occurs in preparations with G III removed, is derived from motoneurone pacemakers, while pattern 2*a*, which is often accompanied by impulse synchronization between spiracles 1 and 2 and never occurs in the absence of G III, may be produced by a command inter-neurone arising in G III and synaptically activating the motoneurones of spiracles 1

and 2; it may simultaneously suppress the activity of their pacemakers. Pattern 2*b* may sometimes be a mixture of *a* and *c* resulting from low activity in the command interneurone, or due to the effect of irregular suppression of pacemaker activity by inhibitory fibres. The spiracle motoneurones therefore seem to act either as segmental oscillators whose frequency can be adjusted, or as follower cells which are driven by a dominant oscillator in G III.

Electrical stimulation of the command interneurones

As an alternative approach electrical stimulation has been applied to split bundles of the connectives between G I and G II, after removal of G III, in an attempt to activate the command interneurones and examine their effect on the segmental pacemaker activity. As previously mentioned the technique suffers from the major defect that the same fibres cannot be stimulated in successive preparations and that unavoidably a mass of axons is excited by each shock. Thus responses may be brought about via the natural pathways or by the activation of large numbers of axons none of which acts as the spiracle command fibre in the intact insect. However, the failure to affect the spiracle motoneruones by the simultaneous stimulation of large numbers of lateral afferent nerves, and the different effects of stimulation of different bundles of split connectives, suggest that massive and non-specific input does not account for the observed results.

Rowell (1964) has shown that dissociation of parts of the central nervous system of a locust drastically modifies the threshold of some reflex activities and the following preparations were therefore employed in the experiments:

1. Whole nervous system intact.
2. Central nervous system intact: all lateral thoracic nerves cut.
3. Connectives between G II and G III cut; lateral nerves of G I and G II either all cut or left intact.
4. Connectives between G I and G II cut; G I lateral nerves all cut or intact.
5. All the above preparations decapitated.

With the exceptions to be mentioned below, the choice of preparation did not affect the results. Although decapitation has marked effects on pacemaker activity, all the responses to connective stimulation could be obtained with or without the head. It was found most convenient to employ preparation 3 in which the frequency of free running could be kept low by the maintenance of hypoxic conditions in the head. Delicate control of the frequency was obtainable by opening and closing the cut ends of cephalic tracheae or by perfusion with hypoxic gases.

Single shocks applied either to a whole connective between G I and G II, or between G II and G III, or to some bundles of the split connectives between G I and G II, produced a 1:1 response in both axons of spiracles 1 and 2 (Fig. 10). Assuming that the natural route has been invoked, excitation of spiracle 2 probably takes place antidromically when stimulation is applied to the G I–II connective but orthodromically when applied to the G II–III connective. The simultaneous excitation of both spiracles by G I–II stimulation suggests the presence of a single command interneurone supplying both, an hypothesis already postulated to account for impulse synchronization between these spiracles. By stimulating at either end of the connective a conduction speed for the interneurone of 0·7–1·0 m./sec. can be measured. With

conduction in the spiracle motor axons taking place at 1·1–1·5 m./sec. and a total latency of 12–15 msec. (Fig. 13), a ganglionic delay of 4–7 msec. can be postulated. This suggests the existence of at least one synapse. Since conduction velocity is probably slowed by the use of petroleum jelly, the central delay may be slightly shorter. Knights (1965) has pointed out that in dragonfly larvae an increased intensity of stimulation reduces central delays, part of the effect being due to spatial summation and part possibly to greater stimulus spread. Slight reductions of latency were obtained by increasing the intensity in spiracle motoneurone preparations, and with very high intensities it was possible to stimulate the motoneurones directly (Fig. 12*c*). High frequencies of stimulation on the other hand increased the latency, presumably due to synaptic fatigue as was found by Knights. Occasionally at low intensities of stimulation the latency was as great as 50 msec., and exceptionally 115 msec., when polysynaptic pathways were probably involved.
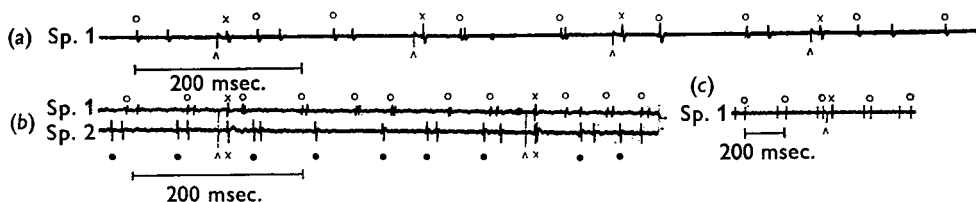

Fig. 10. Records from the spiracle nerves of *Schistocerca* with G III removed, during the stimulation of a bundle of the split connective between GI and G II with single shocks (∧). The stimulated spikes are interpolated into free running activity with little or no perturbation of the rhythm. In (*a*) and (*c*), records are from the spiracle 1 nerve; in (*b*) from the nerves of spiracles 1 and 2. The spontaneous firing of one unit is marked by ○ or ● ; stimulated impulses are marked ×.

Single shocks sometimes gave rise to responses which were interpolated into the free-running rhythm of spiracle 1 (in preparations without G III) hardly affecting the timing of natural spikes in either axon. Examples are shown in Figs. 10 and 11. In other preparations, however, where different bundles of axons were stimulated, the evoked response was sometimes followed either by a temporary acceleration of free running, or by a post-excitatory depression which lasted for twice the normal spike interval. 'Inhibitory' effects of this nature are discussed further below.

In preparations 1 and 2, with G III intact, a similar 1:1 response could be obtained during the expiratory phase when the motoneurones were naturally active, but stimulation failed to produce a response during inspiration when the units remained silent. Thus when the activity of the motoneurones is inhibited by G III during inspiration, electrical stimulation is without effect; but when inhibition is produced as a result of hypoxic conditions in the head, in preparations without G III, electrical stimulation is still effective. The inhibitory command interneurone from G III must therefore act both on the pacemakers and at the synaptic regions of the cells. Head hypoxia, however, affects the pacemakers alone and thus does not prevent the spiracle from making movements synchronized with ventilation under conditions of respiratory stress, since it can still be driven by G III.

In a few preparations stimulation of small bundles of the connective produced double firing in each axon, the spikes in each couplet being 10–15 msec. apart (Fig. 12*f*),

at a time when the normal spike interval was 80 msec. Such double firing is reminiscent of that which sometimes occurs in flight motoneurones of the locust where Wilson (1964) has recognized an intrinsic refractory oscillatory mechanism. Double firing in response to a single input is also a feature of some *Aplysia* ganglion cells when they are fatigued or hyperpolarized (Tauc, 1962). Paired impulses occur in natural trains during pattern 2*a* firing in spiracle motoneurones of the locust, but it is not known if they also arise from a single input (cf. Hoyle, 1959). Electrical stimulation may of course be able to produce them by the activation of two central paths with different conduction rates.
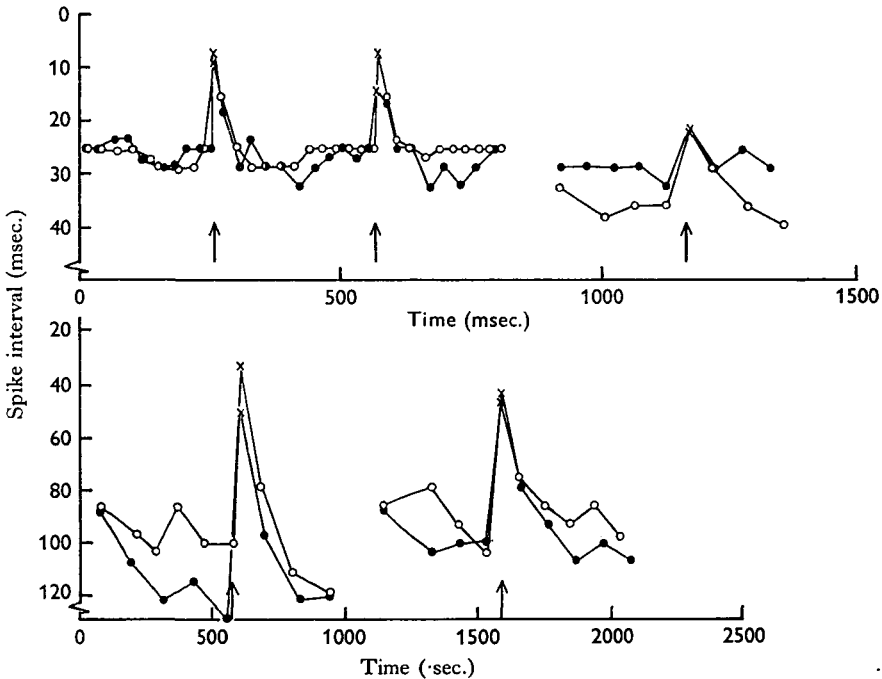

Fig. 11. Plot of consecutive impulse intervals for each free-running unit separately of the spiracle 1 nerve (O—O; ●—●) in *Schistocera* with GIII removed. Single shocks (arrows) are given to a bundle of the split connective between GI and GII, and they interpolate impulses (×) into the spontaneous rhythm without re-setting it. Compare with Figs. 7 and 10.

Threshold stimulation of the connective produces either a response in both spiracle motoneurones or in neither. A response in one alone can be invoked only when its partner is in the refractory period of a preceding P spike. Normally the two units fire synchronously in response to stimulation, but in some preparations their latencies may vary by as much as 20 msec. even when pacemaker activity is suppressed (Fig. 12*d*, *e*). The command interneurone may synapse with both motoneurones, but if only one is caused to fire by a command its activity may in turn excite the other. No such interaction evidently occurs between the pacemaker zones.

Inhibitory effects. By the stimulation of different split bundles of the connective between GII and GIII, several 'inhibitory' effects have been produced. In a few preparations in which low-intensity shocks were delivered at 5/sec., the complete inhibition of free running was obtained in the absence of any other response. An increase in intensity produced a 1:1 response and then with a further increase the

response was followed by a high frequency of free running. A gradual reduction of intensity produced the same sequence in reverse.

Stimulation at about 40 shocks/sec may produce a 1:1 response with every second shock, but after several seconds the response gradually disappears as the synapse fatigues. After a few seconds rest it can again be obtained. At 50/sec. no response is obtained, free running is inhibited and the nerves remain silent (Fig. 14); inhibition may outlast the period of stimulation by several seconds.

High-frequency stimulation may produce a cessation of synaptic responses as a result of post-excitatory depression or fatigue and this may also affect the pacemaker zone. Low-frequency stimulation (5/sec) at low intensities, however, probably causes inhibition by the activation of a specific inhibitory command fibre. It has not so far been possible to obtain in one and the same preparation separate connective bundles whose stimulation produces either excitation alone or inhibition alone, although each has been obtained on different occasions.
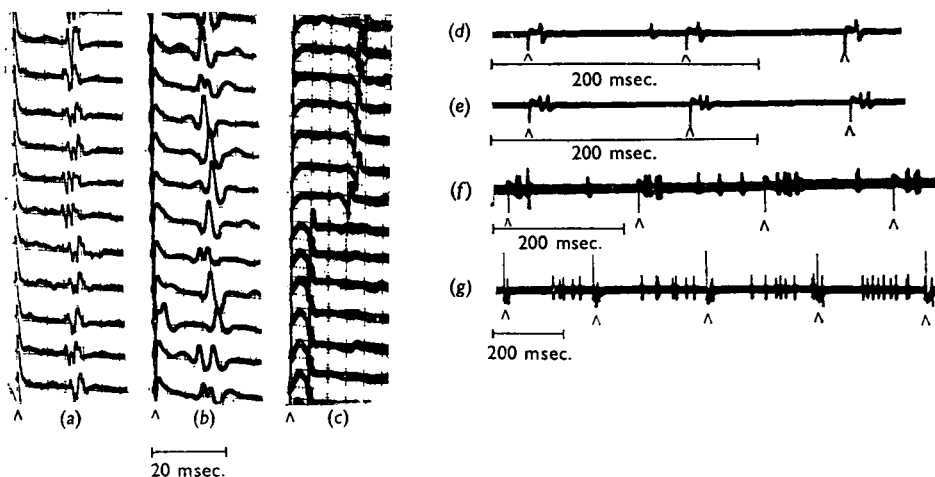

Fig. 12. Records from the transverse nerves of spiracles 1 and 2 during the stimulation of a bundle of the split connective between G I and G II with single shocks (∧) in preparations with G III removed. (*a*) 1:1 responses in the spiracle 2 nerve, both axons firing after a latency of about 16 msec.; the shock artifact starts the sweep. (*b*) The same for spiracle 1 which responds after a similar latency. (*c*) The same for spiracle 1 during a gradual increase of stimulus intensity (read from top to bottom). The latency is reduced slightly at higher intensities and a further increase in intensity then abruptly reduces it to about 5 msec. as the motoneurones are stimulated directly. (*d*) 1:1 responses to shocks delivered at 8/sec.; free running is suppressed and the units respond synchronously. (*e*) As in (*d*) but the two units fire 5 msec. apart. (*f*) Double firing in response to single shocks at 5/sec.; in each couplet the spikes are about 15 msec. apart and free running starts after a further 60–90 msec. (*g*) Shocks at 4/sec. produce a 1:1 response followed by a depression lasting more than twice the normal pacemaker cycle.

When stimulation is repeated on preparations with G III intact, the stimulation of one G I–II connective at 50/sec., the other being intact, produces again a complete inhibition of all activity in spiracles 1 and 2. In addition it induces a marked acceleration of ventilation and probably excites the flight motoneurones (cf. Wilson, 1964). Inhibition of spiracle 2 may therefore be brought about by the normal mechanism which operates during flight, but that of spiracle 1, which remains active in flight (Miller, 1960*b*), is possibly the result of stimulation of an inhibitory interneurone.
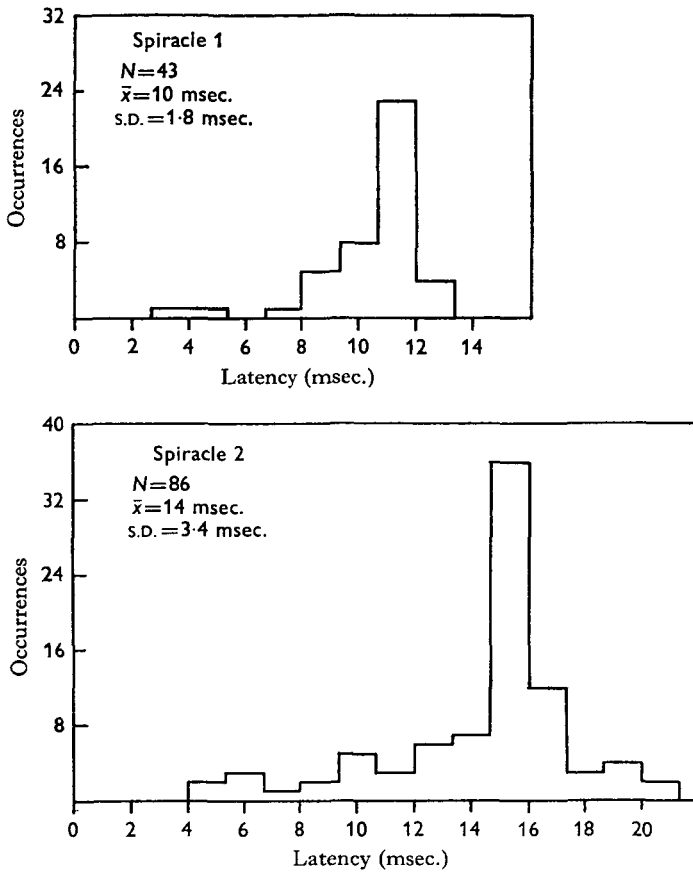
Fig. 13. Histograms of the latencies of the first response of spiracles 1 and 2 when shocks are given to a bundle of the split connective 1 mm. from G I and 3 mm. from G II. The latency includes cord and transverse nerve conduction times as well as synaptic delays.
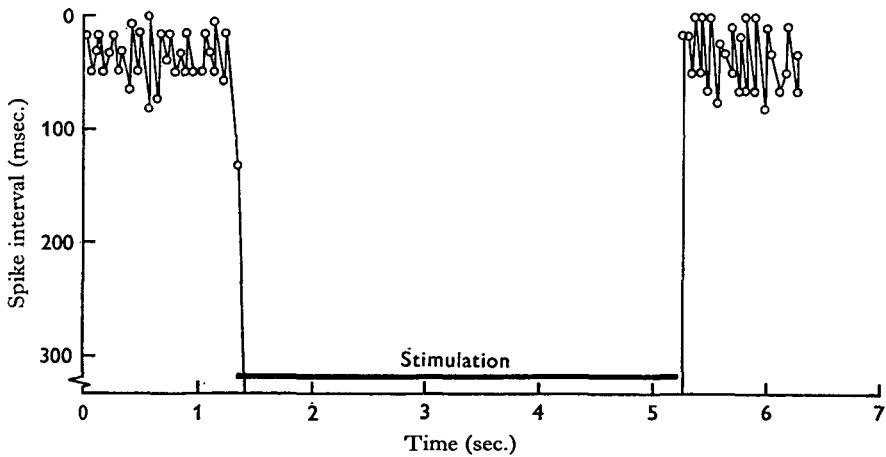

Fig. 14. Plot of consecutive impulse intervals of both units of spiracle 1 before and after inhibition produced by stimulation of the connective between G I and G II at 50 shocks/sec. G III has been removed.

The acceleration of free running. Following stimulation at high or low frequencies there is often a prolonged period during which free running continues at an enhanced frequency; it may last for more than 15 min. and can occur in preparations with or without the head (Figs. 15 and 16). Stimulation of bundles of the split G I–II con-
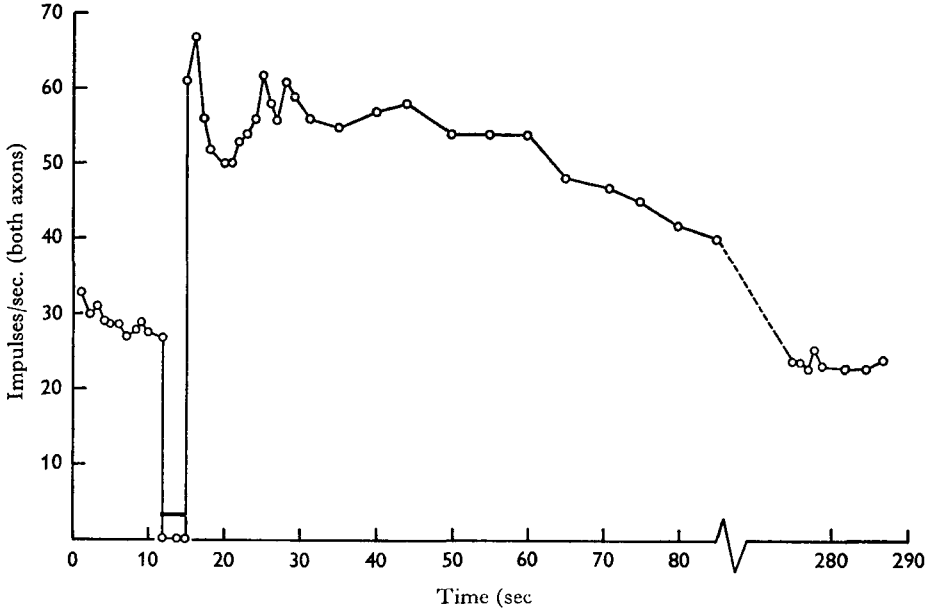

Fig. 15. Plot of the frequency of impulses (both axons) against the time before and after a brief inhibition (bar) produced by connective stimulation as in Fig. 14, showing the gradual return to the original frequency which takes several minutes.
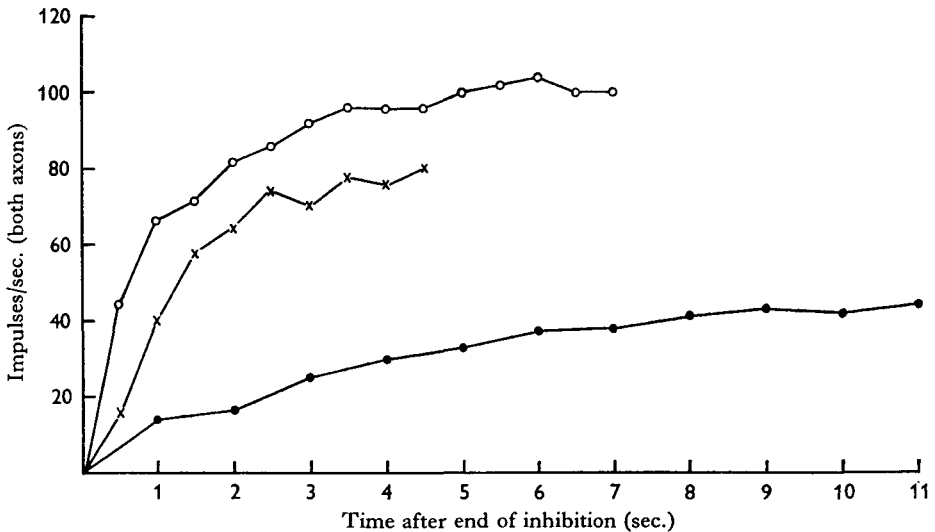

Fig. 16. Three plots of the frequency of impulses (both axons) in the spiracle 1 nerve against time after the end of a brief inhibition produced as in Figs. 14 and 15. Free running gradually increases in frequency over several seconds and subsequently returns to the original frequency (not shown).

nective has occasionally produced acceleration with no other effect. This can take place via G II, since it will also occur in the spiracle 1 motoneurones after section of the connective between stimulating electrodes and G I. Stimulation of the lateral nerves of G I does not produce an enhancement of frequency. Comparable long-lasting effects were found in cockroach ganglia by Luco (1963) after electrical stimulation, and by Kennedy and Preston (1963) in crayfish ganglion cells.

The effects of the gradual application of a block to the G I–II connective

In order to distinguish between the activities of the postulated command interneurones, one connective between G I and G II was progressively blocked either by mechanical damage or by cooling. The other connective was previously cut and the changes in activity of the spiracle 1 nerve were examined. A mechanical block was

Table 1. *The five deviations from the normal firing plan of the spiracle 1 motoneurones to the closer muscle which are produced by the gradual application of a cold block to one connective between G I and G II, the contralateral connective being cut*

	1	2	3	4	5
Expiratory pattern	Normal	No high-frequency burst	Gradual replacement of pattern 2a by 2c	As in 3	In hypoxic preparations, gradual disappearance of all activity
Inspiratory pattern	Free running	Normal	Normal	Gradual appearance of free running	

applied by cutting in turn each bundle of a split connective. The cold block was applied by lifting the connective on a metal hook which was cooled by evaporating ether under reduced pressure in a closed vessel. Such treatment produced a complete block of all activity but was fully reversible when the hook was allowed to return to room temperature. It was thus possible to apply the block several times to the same preparation. Some difficulty in the interpretation of the results is encountered since ventilation was modified considerably by the treatment and this must be distinguished from the selective inactivation of interneurones which supply the spiracle motoneurones.

Five deviations from the normal firing plan were observed during the gradual application of a block, although not all in the same preparation; they are shown in Table 1.

No. 2 (in Table 1) probably results from the general slowing of ventilation which is produced by the block, while nos. 4 and 5 are likely to be the result of the complete loss of contact between G I and G III. However, nos. 1 and 3 are firing plans which are peculiar to this treatment and have not been seen under different circumstances. No. 1 may be explained by the selective block of an inhibitory interneurone and no. 3 by that of an excitatory interneurone which produces pattern 2a.

DISCUSSION

The spiracle motoneurones represent a system which is to some extent functionally independent of the ganglia in which they are found. There is no evidence for diffuse connexions of the type which Fielden (1960) has described for motor units in the

ganglia of the dragonfly abdomen. Nevertheless, their activity is subject to considerable variation and different motor patterns may be used on different occasions to achieve a maintained closure of the spiracle. Similarly, Ewing & Manning (1966) found that in different individuals of the same species of cockroach, and in different species, similar leg movements were not always achieved by the same motor patterns.

The activity of the spiracle motoneurones is compounded from two sources, the segmental and the metathoracic ganglion, and it may be further affected by head ganglia. The contribution of the segmental ganglion (GI for spiracle 1) is a regular smooth pattern of firing, usually independent in the two units, brought about by the activity of pacemakers which lie in some part of the motoneurone. The metathoracic ganglion drives the motor units via command interneurones and in doing so may suppress the activity of the pacemakers. This driven activity comprises a high-frequency burst (pattern 1) at the beginning of expiration and a maintained barrage of firing for the remainder of the stroke (pattern 2a). The regularity of firing and smooth decline in frequency of the high-frequency burst suggest that there is a sudden and maintained depolarization of synaptic regions in both units which produce the rapid discharge in spike-initiating zones with pacemaker properties, possibly some way from the synapses. Spiracles 1 and 2 appear to be controlled independently since either alone can produce a high-frequency burst, or it can occur in both at different frequencies. Pattern 2a, which often follows the burst, comprises regular trains of impulses usually synchronized in the two axons and sometimes occurring in couplets in each. Such activity is often synchronized, impulse for impulse in spiracles 1 and 2, suggesting that one command interneurone excites both on a 1:1 basis. Antidromic stimulation of the transverse nerve does not affect this firing plan and local pacemaker action seems to play no part at least in the establishment of the major periods. Such an interneurone may fire at 20/sec. when the overall frequency in each motor axon is 40/sec., if the second spike of each couplet occurs as a result of an oscillation within the motoneurone. Wilson (1964) has shown this to be the case in the double firing of flight motoneurones, but the evidence is inconclusive for the spiracles.

Electrical stimulation of split bundles of the GI–II connective can produce synchronized impulses in each of the four axons which supply the closer muscles of spiracles 1 and 2. Motoneurones supplying the opener muscle of spiracle 1 do not respond in this way. Inhibitory effects and acceleration of pacemaker activity have also been achieved, but these cannot be assigned with certainty to the activation of specific command interneurones. Low frequencies of stimulation can inhibit pacemaker activity; at higher intensities there is often a 1:1 excitation, whereas higher frequencies of stimulation abolish all activity. Different effects are produced in successive preparations presumably because different combinations of axons are included in the split bundles of the connective. In some preparations 1:1 spikes produced by stimulation of the cord are interpolated into the free-running cycles of the pacemakers without affecting their timing. This suggests that the synaptic region and pacemaker zone may be in different parts of the motoneurone, perhaps in different axon branches, and that the pacemaker is not invaded by synaptically derived spikes; there may thus be two spike-initiating regions. Comparable functional separation of the different regions of the motor units and interneurones in the central nervous system of the crayfish has been demonstrated by Takeda & Kennedy (1965) where they state that the axon

branches 'take on almost the integrative role of separate internuncial elements'. A similar situation has been demonstrated by Tauc & Hughes (1963) in certain *Aplysia* neurones where not all branches of an axon are invaded by a spike. In other locust preparations, however, 1 : 1 responses in the spiracle nerves may be followed by temporary depressions before normal pacemaker firing is resumed. This may result from the simultaneous stimulation of excitatory and inhibitory interneurones, the latter with a slower conducting speed, which are both included in some bundles but not in others. But alternatively there may be weak interaction between synaptic and pacemaker regions, and differences between preparations may sometimes depend on their ages or on the extent of damage to tracheae during dissection. The possible effects of stimulus spread acting directly on synapses cannot be excluded.

Pattern 2 c, like free running, comprises regular and independent firing in the two motoneurones and it is probable that G III plays no part in its production beyond exercising a general effect on the frequency. Such activity can be re-phased by antidromic stimulation of the transverse nerve before or after the removal of G III and is quite independent in adjacent spiracles.

In adult dragonflies spiracle closure is maintained by a pattern of firing in the two axons which closely resembles free running in the locust. Activity corresponding to pattern 2 a has not been seen and it appears that the dragonfly lacks the interneurone responsible for this pattern (Miller, 1965). The presence of an excitatory command interneurone responsible for pattern 2 a in the locust may be explained by the need to maintain spiracle closure at times when free running is slowed by hypoxic conditions in the head. Such activity will then permit abdominal pumping to maintain an efficient through-conduction of air and prevent ventilation from becoming tidal, which probably happens in dragonflies under such conditions.

A tentative model for the arrangement and connexions of one motoneurone supplying the closer muscle of spiracle 1 is shown in Fig. 17. It is depicted with separate pacemaker and synaptic regions and with two inhibitory and three excitatory interneurones which make synaptic connexions. The interneurone responsible for pattern 2 a is shown as synapsing in a separate region of the motoneurone from that which produces the high-frequency burst of pattern 1; the latter is shown running to the pacemaker zone, but it may equally well act through a third discrete part of the cell which is able to initiate spike trains in response to a maintained depolarization. Several dozen different models could be drawn all accounting for the observed properties; the virtue of the present one is only that it provides a terse summary of the findings to date. It may be compared with the diagram of the neurone of the cardiac ganglion which is reproduced as Fig. 3·4 by Bullock & Horridge (1965). Indeed the spiracle motoneurones are comparable in several ways with the follower neurones of the crustacean cardiac ganglion (Maynard, 1955). Both are capable of spontaneous activity when isolated from the principal pacemaker (for the spiracle this resides in G III), but in both such spontaneous activity may seldom appear in the intact animal. Bullock (Bullock & Horridge, 1965, p. 319) has suggested that such spontaneity may represent 'a way of poising the cells so close to threshold that they will follow: (*a*) with a small synaptic input, or (*b*) with a short delay, or (*c*) in conjunction with the property of noninvasion of the soma and pacemaker potential regions by the spikes...with a high frequency, repetitive burst, or (*d*) any combination of the three.' In the spiracle

motoneurone such spontaneity *is* expressed at times, and can in some species con-
tribute to the motor output, as well as possibly maintain the cell in a poised state of
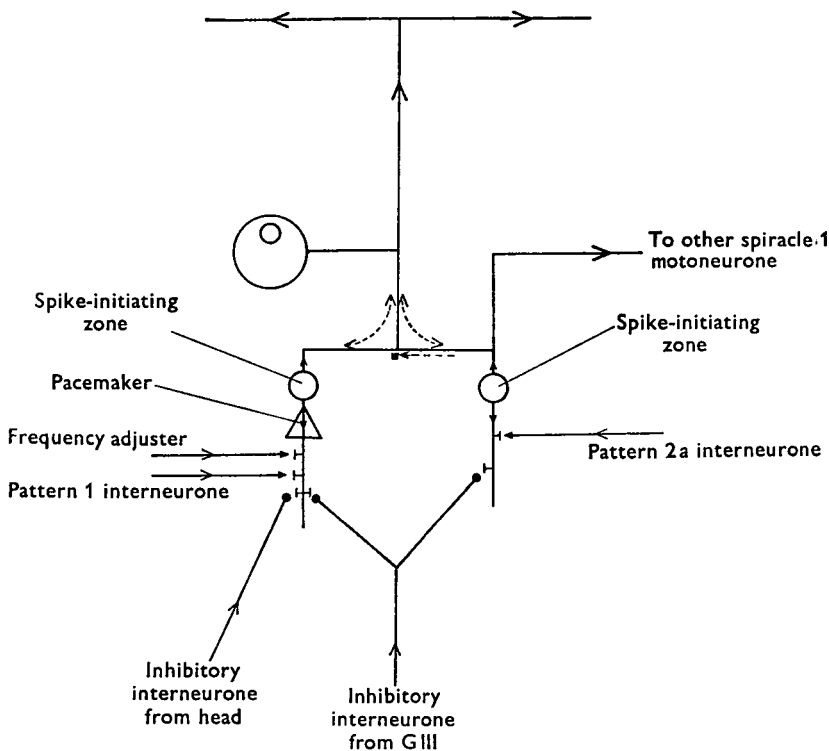readiness.

Fig. 17. Model of the hypothetical lay-out and connexions of one motor unit supplying the
closer muscle of spiracle 1. Explanation in the text.

SUMMARY

1. Three principal motor patterns have been distinguished in the nerves to the
closer muscle of spiracle 1 of the locust. Pattern 1 brings about muscular contraction
and spiracle closing; pattern 2 maintains the closure and may be brought about either
by the activity of pacemakers located in each motoneurone or by synaptic activity or
by a mixture of both. The possible origins of pattern 2 activity have been located by
antidromic stimulation and by observations on the occurrence of impulse synchron-
ization between the units of one segment and those of adjacent segments. In pattern
3, firing slows or stops and the spiracle opens.

2. In most individuals of *Locusta*, pacemaker activity predominates in pattern 2
firing, while in *Schistocerca* synaptic activity is more common. Different individuals
of either species may remain consistent to one firing plan for long periods; their effect
at normal frequencies at the muscle is indistinguishable.

3. Electrical stimulation of split bundles of the connectives has gone some way
to distinguishing between the activity of possibly three command interneurones
which run from the metathoracic ganglion and synapse with the spiracle motoneurones.
These produce 1:1 excitation, acceleration of pacemaker activity or inhibition of the

pacemakers, and in the intact insect are believed to bring about accurate synchronization of the spiracle movements with abdominal pumping.

REFERENCES

BIEDERMAN, M. A. (1964). Response of spontaneous units in crayfish ventral cord to direct current. *Comp. Biochem. Physiol.* **12**, 311–30.

BIEDERMAN-THORSON, M. A. (1966). Some mechanisms for unit activity in isolated crayfish central nervous systems. *J. gen. Physiol.* **49**, 597–612.

BULLOCK, T. H. & HORRIDGE, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates.* San Francisco and London: W. H. Freeman and Co.

EWING, A. W. & MANNING, A. (1966). Some aspects of the efferent control of walking in three cockroach species. *J. Insect Physiol.* **12**, 1115–18.

FIELDEN, A. (1960). Transmission through the last abdominal ganglion of the dragonfly nymph. *J. exp. Biol.* **37**, 832–44.

FIELDEN, A. & HUGHES, G. M. (1962). Unit activity in the abdominal nerve cord of a dragonfly nymph. *J. exp. Biol.* **39**, 31–44.

HAGIWARA, S. (1961). Nervous activities of the heart in Crustacea. *Ergebn. Biol.* **24**, 287–311.

HOYLE, G. (1959). The neuromuscular mechanism of an insect spiracular muscle. *J. Insect Physiol.* **3**, 378–94.

HUBER, F. (1960). Experimentelle Untersuchungen zur nervosen Atmungsregulation der Orthopteren (Saltatoria, Gryllidae). *Z. vergl. Physiol.* **43**, 359–91.

IWASAKI, S. & WILSON, D. M. (1966). Neuropile potentials following wing sensory nerve stimulation in locusts. *Comp. Biochem. Physiol.* **17**, 693–5.

KENNEDY, D. & PRESTON, J. B. (1963). Post-activation changes in excitability and spontaneous firing of crustacean interneurones. *Comp. Biochem. Physiol.* **8**, 173–9.

KNIGHTS, A. (1965). The activity of single motor fibres in arthropods. I. The dragonfly nymph. *J. exp. Biol.* **42**, 447–61.

LUCO, J. V. (1963). Plasticity and the natural response of a nervous organization. In *Perspectives in Biology* (eds. C. F. Cori, V. G. Foglia, L. E. Leloir and S. Ochoa), pp. 355–60. Amsterdam.

MAYNARD, D. M. (1955). Activity in a crustacean ganglion. II. Pattern and interaction in burst formation. *Biol. Bull. mar. biol. Lab. Woods Hole* **109**, 420–36.

MILL, P. J. & HUGHES, G. M. (1966). The nervous control of ventilation in dragonfly larvae. *J. exp. Biol.* **44**, 297–316.

MILLER, P. L. (1960a). Respiration in the desert locust, II. The control of the spiracles. *J. exp. Biol.* **37**, 237–63.

MILLER, P. L. (1960b). Respiration in the desert locust, III. Ventilation and the spiracles during flight. *J. exp. Biol.* **37**, 264–78.

MILLER, P. L. (1965). The central nervous control of respiratory movements. In *The Physiology of the Insect Central Nervous System* (eds. J. E. Treherne and J. W. L. Beament), pp. 141–55. London and New York: Academic Press.

MILLER, P. L. (1966). The regulation of breathing in insects. In *Advances in Insect Physiology*, vol. 3 pp. 279–354 (eds. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth). London and New York: Academic Press.

NARAHASHI, T. (1963). The properties of insect axons. In *Advances in Insect Physiology*, vol. 1, pp. 175–256 (eds. J. W. L. Beament, J. E. Treherne and V. B. Wigglesworth). London and New York: Academic Press.

PRESTON, J. B. & KENNEDY, D. (1962). Spontaneous activity in crustacean neurons. *J. gen. Physiol.* **35**, 831–36.

ROEDER, K. D. (1966). Interneurons of the thoracic nerve cord activated by tympanic nerve fibres in noctuid moths. *J. Insect Physiol.* **12**, 1227–44.

ROWELL, C. H. F. (1964). Central control of an insect segmental reflex. I. Inhibition by different parts of the central nervous system. *J. exp. Biol.* **41**, 559–72.

TAKEDA, K. & KENNEDY, D. (1965). The mechanism of discharge pattern formation in crayfish interneurones. *J. gen. Physiol.* **48**, 435–53.

TAUC, L. (1962). Identification of active membrane areas in the giant neuron of *Aplysia. J. gen. Physiol.* **35**, 1099–116.

TAUC, L. & HUGHES, G. M. (1963). Modes of initiation and propagation of spikes in the branching axons of molluscan central neurons. *J. gen. Physiol.* **46**, 533–49.

WILSON, D. M. (1961). The central nervous control of flight in a locust. *J. exp. Biol.* **38**, 471–90.

WILSON, D. M. (1964). Relative refractoriness and patterned discharge of locust flight motor neurons. *J. exp. Biol.* **41**, 191–205.

WILSON, D. M. & DAVIS, W. J. (1965). Nerve impulse patterns and reflex control in the motor system of the crayfish claw. *J. exp. Biol.* **43**, 193–210.

WILSON, D. M. & WEIS-FOGH, T. (1962). Patterned activity of coordinated motor units, studied in flying locusts. *J. exp. Biol.* **39**, 643–67.

WILSON, D. M. & WYMAN, R. J. (1965). Motor output patterns during random and rhythmic stimulation of locust thoracic ganglia. *Biophys. J.* **5**, 121–43.