

SPATIAL AND TEMPORAL CHANGES IN THE COUPLING OF COCKROACH SPIRACLES TO VENTILATION

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INTRODUCTION

Different rhythmical behaviour patterns may sometimes be achieved through alterations of the coupling of motor neurones to an oscillator. Such a statement suggests independence of the oscillator from at least some motor neurones and this seems to be the case in, for example, locust flight (Wilson, 1964; Kendig, 1968) and ventilation (Miller, 1966). A good example comes from some of the motor neurones responsible for warm-up and for flight in large moths, since in the former they act as synergists while in the latter they become antagonists (Kammer, 1968, 1970). The ventilatory and spiracle systems of locusts, mantids and cockroaches offer another example where appropriate alterations of spiracle activity can produce airstreams which are driven either posteriorly or anteriorly through longitudinal tracheae in the ventilating insect. Changes in the direction of ventilating airstreams have been reported in several species of Orthoptera and Dictyoptera (McArthur, 1929; McGovran, 1932; Fraenkel, 1932), but the mechanism of reversal has so far remained undescribed. Reversals are shown here to be achieved by alterations of the timing of the active phase of spiracle motor neurones in the ventilatory cycle. The switch mechanism responsible for the changes in neural coupling to the ventilatory oscillator can be experimentally controlled in the cockroach *Blaberus giganteus*, and this has enabled the forms of activity to be characterized and transitional states to be examined. It has been found that the temporal changes in the coupling mode of the spiracles may be accompanied by spatial changes, since some pairs of abdominal spiracles when coupled to inspiration act symmetrically whereas when coupled to expiration they act asymmetrically. The side producing the major motor output during expiratory coupling can be controlled experimentally. Switch mechanisms of this type are of considerable interest in the study of the neural basis of insect behaviour, and the spiracle system provides a convenient example for their exploration.

MATERIAL AND METHODS

A culture of the large cockroach *Blaberus giganteus* (L.) based on a single female captured in Honduras in 1967 has been kept in the laboratory at Oxford under normal conditions. Observations have been made on adults of both sexes, but males are the more convenient for the recording of spiracle activity. Electromyograms and intracellular recordings from spiracle and ventilatory muscles together with extracellular recordings from motor nerves have been made with conventional techniques (Miller,

1971). Ventilatory movements were recorded with a transducer. Preparations were perfused with known gas mixtures in a plastic gassing box while electrical recordings were made (Miller, 1964). Examinations were carried out during the day when cockroaches are normally quiescent and under cover. Most attention has been paid to the last abdominal spiracle, no. 10.

RESULTS

Ventilatory behaviour. A resting *Blaberus* ventilates intermittently; usually several pumping cycles occur with long intervening quiescent periods. Activity, or perfusion with 5% CO₂, induces more or less continual pumping in which spiracle 1 closes during expiration and spiracle 10 of the left or right side, together with other abdominal spiracles, opens with expiration. A posteriorly directed airstream is probably produced by this activity. In contrast, perfusion with a mixture of nitrogen and air giving less than 5% oxygen produces slower and deeper ventilation; both spiracles 10 and some other abdominal spiracles open during inspiration while spiracle 1 remains open for much of the cycle. This activity probably creates an anterior flow of air through the tracheal system. Spiracle 10 is normally coupled to expiration (opens during expiration) whenever ventilation is fast while with slower deeper breathing (which can be induced either by hypoxia or decapitation) it is coupled to inspiration, opening during the inspiratory stroke. Electrical stimulation of one thoracic connective in a decapitated cockroach with 1 msec shocks at 20/sec causes acceleration of ventilation to a maximum of 6 Hz and a change from inspiratory to expiratory coupling of spiracle 10. Thus the form of the coupling of the spiracles is probably determined by the type of ventilation and not directly by the perfusing gas mixture, but the latter provides a convenient means of manipulating it. The interesting feature is that with inspiratory coupling both spiracles 10 act symmetrically, but during expiratory coupling only one spiracle 10 is active. This is described in more detail below.

In *Sphodromantis lineola* similar changes in spiracle coupling have been observed, with spiracle 1 possibly playing a more active role during anteriorly directed ventilation. Measurements of the transfer of air have been made in a ventilating mantid by Fraenkel's (1932) technique and a photographic recording method, while observing the spiracles directly. They confirm that air moves through in the expected direction, determined by the form of spiracle activity. They also show that reversals of the airstream can occur suddenly and spontaneously and that the volume of air pumped anteriorly can be as great as that pumped in the more usual posterior direction (Fig. 1).

Morphology and innervation of spiracle 10. Spiracle 10 of *Blaberus* opens at the end of a short blunt nozzle close to the cercus. It is equipped with a small closer muscle and a many times larger opener divided into two regions (Figure in Miller, 1973). An elastic cuticular hinge aids the action of the closer and can close the valve fully when the muscles have been removed. Most other known spiracles have elastic mechanisms which assist or replace the opener muscle.

The closer muscle is innervated by two axons which arise in the median nerve of the ganglion in the next anterior segment (the 5th abdominal ganglion). Right and

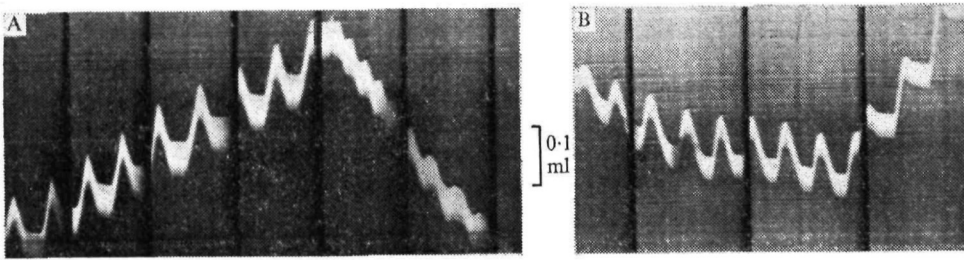


Fig. 1. Photographic records of ventilation in a female *Sphodromantis lineola*. The head and prothorax including spiracle 1 are sealed into a small chamber from which a capillary tube leads out (cf. Fraenkel, 1932). A drop of dark liquid placed in the tube monitors the movement of air through the chamber brought about by ventilation. Its excursions are photographed on moving film. Each oscillation of the white line represents movement of air in and out of spiracles 1 during a ventilatory cycle. Drift of the line upwards represents air pumped posteriorly, entering through spiracle 1, while the downward drift represents air moved anteriorly leaving from spiracle 1. In trace A posterior ventilation suddenly changes to rapid anteriorly directed ventilation. In trace B weakly anterior ventilation suddenly changes to strongly posterior ventilation. Black vertical bars represent 3.8 sec intervals. Vertical scale = 0.1 ml.

left closer muscles receive the same patterns of motor activity, as is known elsewhere (Case, 1957). Left and right openers are separately innervated by paired neurones arising in the 6th abdominal ganglion and they are thus able to act independently of each other. Intracellular records from the opener show that all the fibres penetrated receive dual innervation from a 'slow' axon producing 1–2 mV responses and a large 'fast' axon producing *ca.* 50 mV responses. The activity to be described below is derived almost entirely from the fast axon. The antagonistic muscles of the spiracle are therefore innervated from adjacent ganglia, as also occurs in the abdominal spiracles and ventilatory muscles of a segment in the locust (Lewis, Miller & Mills, 1973).

Patterns of activity in spiracle 10 during expiratory and inspiratory coupling

Continual activity can be recorded in the closer muscle when ventilation ceases and the spiracle remains closed. The frequency of potential changes can be increased by tactile stimulation applied to almost any part of the insect. With inspiratory coupling symmetrical bursts of similar frequency and duration appear in left and right opener muscles and they cause valve opening during inspiration. Each burst, consisting of spikes in the fast axon at 100–150/sec, lasts for up to 350 msec (Fig. 2 C, E) and coincides with cessation of firing in the closers. In contrast, with expiratory coupling an opener burst occurs in only one of the pair of spiracles corresponding to the asymmetrical activity described above. The burst comprises spikes in the fast axon at 300–400/sec, and lasts 100–150 msec; exceptionally there are up to 480 spikes/sec and the burst lasts only 30–50 msec, producing a very brief rapid valve opening during the expiratory stroke (Fig. 2 A, B, D). The spiracle showing the expiratory burst is described as 'dominant', and the other, which may show no activity or only a few spikes at the end (or less commonly at the beginning and end) of the burst of the dominant, is termed the 'subordinate' spiracle. Symmetrical bursts in left and right openers have never been seen during expiratory coupling.

By the use of appropriate changes in the perfusing gas mixtures it is possible to

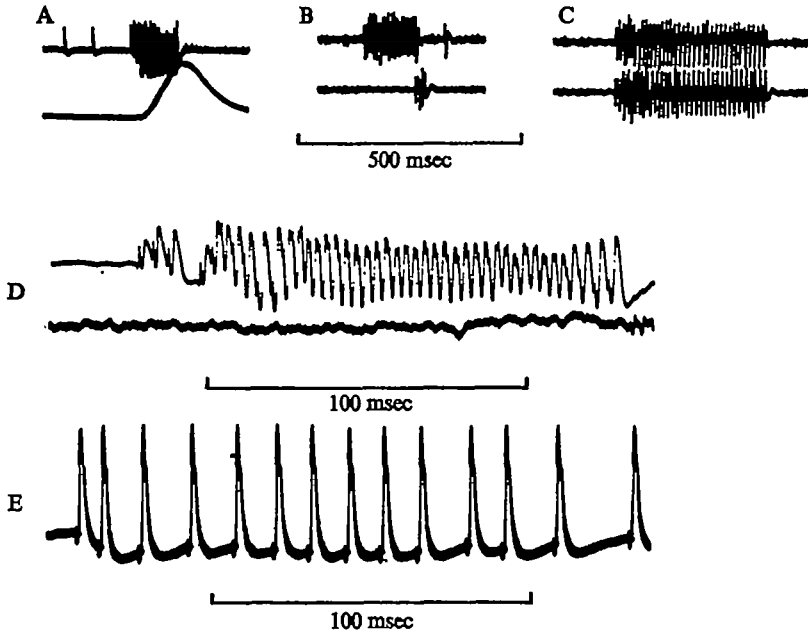


Fig. 2. Electromyograms showing activity in the opener muscle of spiracle 10 resulting from spikes in the 'fast' axon. A, expiratory burst (upper line) and transducer record of ventilation (lower line), expiration upwards. B, dominant opener showing expiratory burst (upper) and subordinate opener showing slight activity (lower). C, symmetrical inspiratory bursts in left and right openers. D, expiratory burst in dominant opener (upper) with nerve spikes and muscle responses separately visible; no activity in subordinate opener (lower). E, part of an inspiratory burst in an opener; nerve spikes and muscle responses are separately visible.

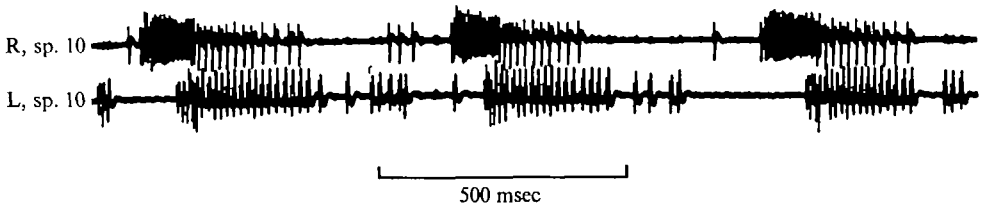


Fig. 3. Electromyograms of activity in opener muscle of spiracle 10 during transitional coupling. The right spiracle (top) is dominant and shows strong expiratory bursts and weak inspiratory bursts; the subordinate left spiracle shows slight expiratory activity and strong inspiratory bursts.

examine transitions between the two forms of coupling. The transition may be complete in 1-2 cycles, or an intermediate state of coupling may persist for many cycles. In the latter case one opener shows an expiratory burst and both then show an ensuing inspiratory burst (Fig. 3); in this way the dominant spiracle may open twice per ventilatory cycle, once with expiration and once with inspiration. During such transitional coupling the dominant opener always shows inspiratory bursts which are shorter and at a lower spike frequency than those of the subordinate opener. This may occur because of post-inhibitory rebound in the subordinate.

In conclusion, bursts of impulses causing valve opening normally appear in spiracle 10 either during expiration or during inspiration. In the former case only

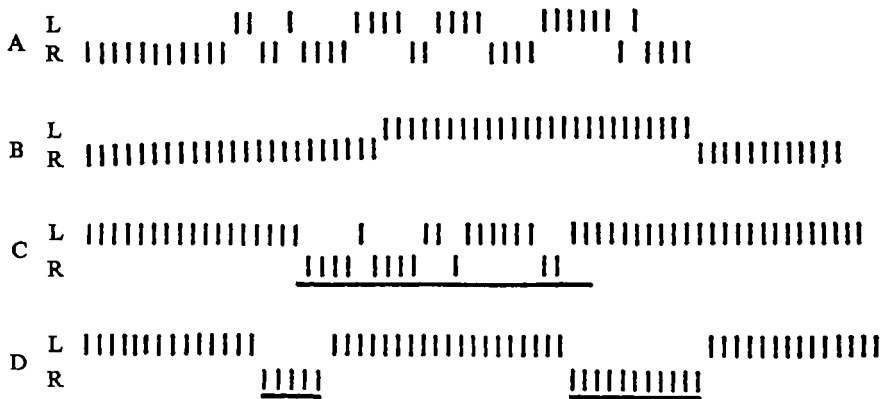


Fig. 4. Diagram showing dominance relationships during expiratory coupling in spiracle 10 in different cockroaches. Each vertical stroke indicates the occurrence of an expiratory burst in left or right spiracle. A and B show spontaneous changes; in B a period of right dominance is followed by a similar period of left dominance, and then a further period of right dominance. C, stimulation of the right cercus of a left-dominant cockroach (horizontal line) produces temporary right dominance. D, electrical stimulation of the right connective between pro- and mesothoracic ganglia (horizontal lines) produces right dominance for as long as stimulation is maintained.

one spiracle of the pair shows the activity, but in the latter they act symmetrically. Maintained transitional states are sometimes seen in which the spiracles act in a 'bipartisan' manner.

Experimental control of the dominant spiracle during expiratory coupling

Unstimulated cockroaches often show persistent dominance on one side. More cockroaches seem to be left than right dominant, but this has not been quantified so far. Others again show spontaneous changes from moment to moment in an unpredictable fashion. Simultaneous recordings of activity in a dorso-ventral ventilatory muscle and in a spiracle 10 opener show that during expiratory coupling changes of spiracle dominance from one side to the other are not accompanied by changes in ventilatory activity of the abdomen. Dominance seems to affect only the spiracles.

The side showing dominance can be controlled experimentally. For example, electrical stimulation of a cercal or leg nerve, or tactile stimulation of cercal or leg receptors, causes the spiracle on the stimulated side to become dominant. The dominance may not persist for more than a few ventilatory cycles, possibly as a result of central habituation (Fig. 4), but the effect, which is best seen following cercal stimulation, can be obtained many times.

Cutting one thoracic connective between pro- and meso-thoracic ganglia makes the contralateral spiracle 10 consistently dominant. However, electrical stimulation of the connective posterior to the cut with 1 msec shocks at 20/sec causes the ipsilateral spiracle to become dominant, and dominance now persists for as long as stimulation is maintained (Figs. 4 and 5). As soon as stimulation ceases dominance reverts to the original side. Connectives have been stimulated in cockroaches with an intact CNS at several points in the abdomen and thorax with similar results. It appears that

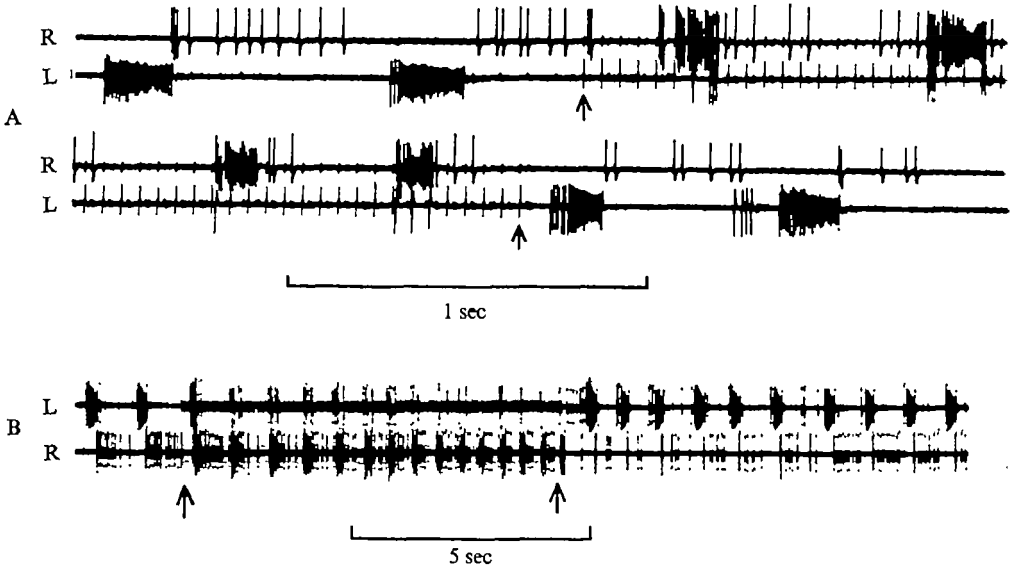


Fig. 5. Electromyograms from the openers of spiracles 10 during stimulation of the right thoracic connective between pro- and mesothoracic ganglia; stimulation is at 20 shocks per second between the arrows. In each case the left spiracle is initially dominant but the right becomes dominant throughout the period of stimulation. The right connective is cut anterior to the stimulating electrodes. A, a continuous record. B additionally shows an acceleration of ventilation during connective stimulation. R, right; L, left.

dominance of the left or right spiracle 10 opener during expiratory coupling depends on asymmetrical activity in the CNS which may result from unilateral sensory stimulation and possibly also from an innate imbalance.

*Possible interactions between motor neurones and interneurones
in the production of asymmetric outputs*

Motor neurones to the opener muscles of spiracles 10 are situated in the last abdominal ganglion. After isolation of this ganglion the fast motor units show irregular persistent activity. However, if the ganglion is divided medially the fast motor neurone in each hemi-ganglion fires at a steady frequency for long periods. To determine whether unilateral dominance resulted from reciprocal inhibitory coupling between the fast opener motor neurones, the 5th and 6th (last) ganglia were both divided medially into equal halves but they were left in contact with the rest of the CNS through their connectives. During inspiratory coupling the patterns of activity in the fast axons were hardly changed by this operation although the spikes appeared more regularly during the burst (Fig. 6A). During expiratory coupling the activity was similar to that in the intact insect, but bursts were shorter than normal (Fig. 6B, C). The main features of the two forms of coupling are unchanged by this operation and dominance can still be established on either side. The asymmetric output does not therefore seem to arise directly from interaction between the motor neurones and probably depends on interneurones situated more anteriorly.

With one connective cut between the 5th and 6th abdominal ganglia a marked asymmetry develops when the spiracles are coupled to inspiration, with short low-

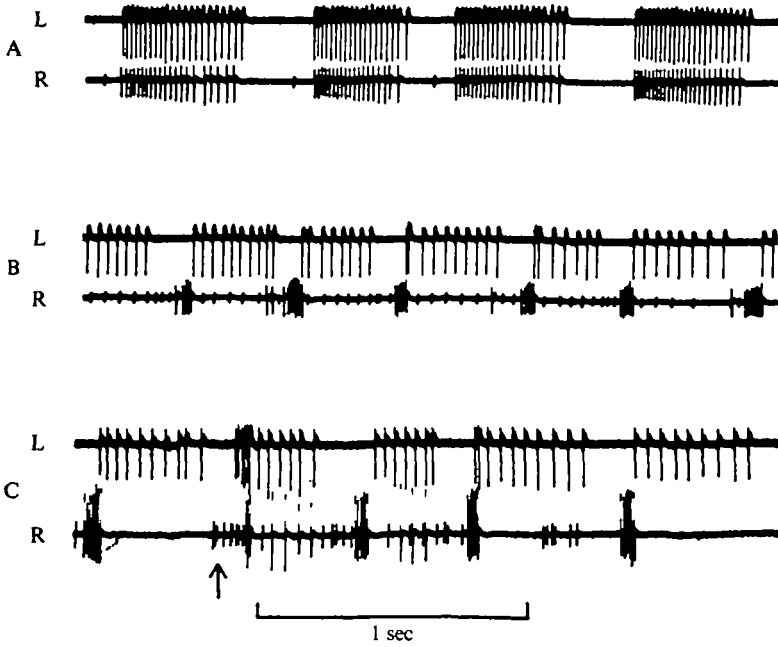


Fig. 6. Electromyograms from the openers of spiracles 10 in a cockroach with the 5th and 6th abdominal ganglia medially cut through but otherwise intact. A, symmetrical bursts during inspiratory coupling. B and C, asymmetrical bursts during expiratory coupling. In B and C the right opener (R) is dominant, but in C the left (L) opener is caused to become dominant for one cycle by stimulation of the ipsilateral cercus (arrow). The small spikes in records from the right spiracle are from the closer.

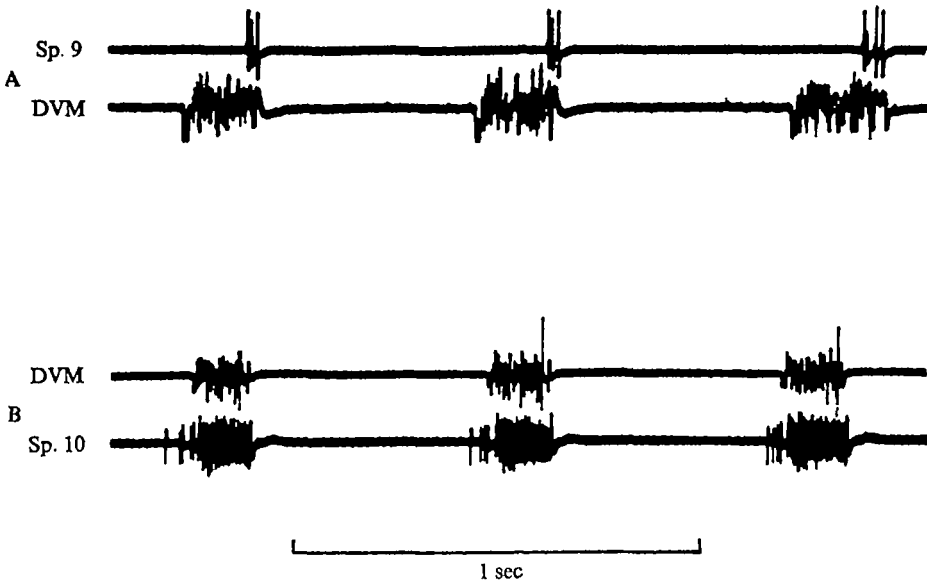


Fig. 7. Electromyograms of activity in a dorso-ventral expiratory muscle (DVM) and a spiracle opener muscle. A, expiratory bursts in spiracle 9 occur at the end of the DVM expiratory burst following mechanical stimulation of the insect. B, expiratory bursts in the opener of spiracle 10 more or less coincide with those in the DVM; this is so only during rapid ventilation.

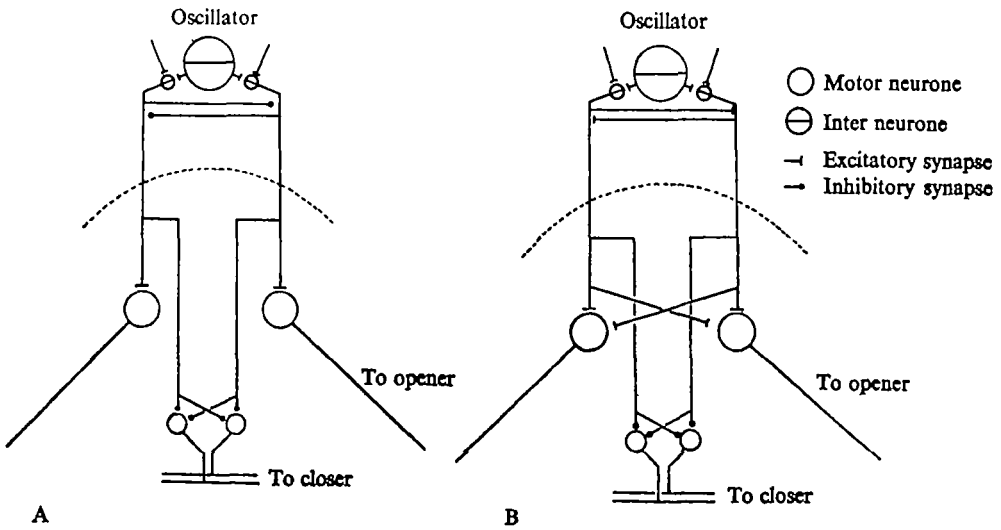


Fig. 8. A model to explain the forms of coupling of the motor neurones supplying spiracle 10 to the ventilatory oscillator. In A, the oscillator, possibly situated in the metathoracic ganglion, drives two coordinating interneurons during the expiratory phase and these in turn cause burst formation in one of the two 'fast' opener motor neurones, and simultaneously inhibit activity in the two closer motor neurones. The coordinating interneurons have reciprocal inhibitory connexions. Each receives input which when unequal biases the output of one so that it fires more strongly and suppresses the output of the other; this in turn establishes the dominant spiracle. The system is unstable, and when input is equal on the two sides an inherent asymmetry may determine the dominant spiracle. In B, the oscillator is shown connected to a different set of coordinating interneurons which are activated during the inspiratory phase and produce symmetrical activity in the openers while inhibiting the closers. They are positively coupled and this helps to overcome any tendency to act asymmetrically. They also have weaker effects on the contralateral opener.

frequency bursts appearing on the damaged side. Their occurrence suggests either that the opener motor neurones are positively but weakly coupled to each other, or that a co-ordinating interneurone drives the ipsilateral motor neurone strongly, but the contralateral one weakly.

The activity of other abdominal spiracles. Spiracles 4, 5 and 9 are associated with extensive glandular regions surrounding the tracheae close to the spiracles (cf. Roth & Eisner, 1962). The tracheal branch joining the spiracle to the longitudinal trunk is long and looped anteriorly in these spiracles, whereas it is short and broad in others. Spiracles 4, 5 and 9 usually remain closed during hyperventilation produced by perfusion with CO_2 or with nitrogen-rich gas mixtures, whereas if the insects are stimulated mechanically they become active for a few cycles, opening very briefly towards the end of each expiratory stroke (Fig. 7). They have not been observed to open with inspiration, nor is their activity markedly asymmetrical. When handled *Blaberus giganteus* emits a strong odour and the activity of these spiracles would seem to be well suited to ejecting a volatile chemical which might act to deter predators. Such is known to happen in some other cockroaches, for example, in spiracle 4 (second abdominal) of *Diploptera* (Roth & Stay, 1958), but the function of the glandular secretions of *Blaberus* is unknown.

Spiracles 3, 6, 7 and 8 show behaviour similar to that of spiracle 10, but they appear

to have higher thresholds, and bursts recorded from their opener muscles are usually shorter and at a lower spike frequency. They show asymmetrical activity when coupled to expiration.

DISCUSSION

Spiracles 10 and other abdominal spiracles of *Blaberus* open with expiration or with inspiration. This activity probably helps to drive air currents either posteriorly or anteriorly through longitudinal tracheal trunks. Changes in the coupling of the spiracle motor neurones to the ventilatory oscillator account for the changes in activity, and such changes can be experimentally controlled by the use of appropriate gas mixtures. In *Schistocerca* (Miller, 1966) and in *Periplaneta* (Farley, Case & Roeder, 1967) the major ventilatory oscillator is believed to lie in the metathoracic ganglion. Assuming this to be true for *Blaberus*, changes in spiracle activity could be accounted for by one of three possible mechanisms: (1) alterations of the coupling of *one* set of co-ordinating interneurones to the ventilatory oscillator in the metathoracic ganglion, so that they were activated either during expiration or during inspiration, could take place under the influence of appropriate command cells. (2) one of *two* sets of coordinating interneurones, one coupled to inspiration and the other to expiration, could be driven by the ventilatory oscillator, again the appropriate set being selected by command cells. (3) changes in coupling could take place at the level of the motor neurones and not at that of interneurones.

The first possibility is unlikely since the asymmetrical output which is characteristic only of expiratory coupling does not depend on interactions between the motor neurones and is determined more anteriorly at the interneuronal level. The third possibility is difficult to exclude but it demands additional interneurones to operate the switches in each ganglion and does not readily explain the maintenance of asymmetrical expiratory coupling after medial longitudinal section of ganglia. The second possibility agrees with the data but clearly several alternative schemes could be devised. In the model shown in Fig. 8A a pair of coordinating interneurones with reciprocal inhibitory connexions can be coupled to the expiratory phase of the ventilatory oscillator to produce asymmetric expiratory spiracle activity. A second pair of interneurones (Fig. 8B) can be activated by the inspiratory phase of the oscillator and causes symmetrical inspiratory activity of the spiracle valves. The second pair has in addition a weak effect on the fast opener motor neurone of the other side. Transitional types of coupling are accounted for by the simultaneous coupling of both sets of interneurones to the oscillator. Coupling is controlled by two sets of command fibres which produce appropriate biases on the oscillator-to-co-ordinating-interneurone synapses and which themselves are mutually inhibitory when strongly active; transitional states persist therefore only during weak command-fibre activity. The side of the dominant spiracle during expiratory coupling is determined by unequal activity in the left and right command fibres, and this in turn may reflect greater sensory input on one side. The coordinating interneurones which link the spiracles to inspiration have a reciprocal excitatory action on each other and this counteracts any imbalance caused by unilateral sensory input and so produces a symmetrical output.

Expiratory coupling seems to involve a system in which weak asymmetrical input

to the ventilatory mechanism results in strong asymmetrical output to certain abdominal spiracles. There may in addition be an inherent asymmetry producing the persistent left or right dominance of some cockroaches which cannot be accounted for by peripheral input. Inherent asymmetries have been described in many other insect systems, for example in *Oncopeltus* walking (Chapple, 1966; Wilson & Hoy, 1968) and in the locust flight system (Wilson, 1968). However, they are believed to be corrected for in these examples by sensory input, whereas in cockroach spiracles the system is unstable as in a flip-flop. In sphingid pupae gin traps close either to the left or to the right in response to mechanical stimulation (Bate, 1972). In this system, too, weak asymmetric inputs are converted into strong asymmetric outputs.

The function of asymmetrical activity during expiratory coupling in cockroach spiracles is unknown. Spiracles 1 of *Schistocerca* and *Blaberus discoidalis* each receive a paired motor axon in addition to axons from the median nerve system (Miller, 1969) and thus are capable of unilateral action, but this does not normally seem to occur. However, unilateral action has been detected in spiracle 4 (second abdominal) of *Gromphadorhina* by Burns (pers. com.) and it allows the insect to squeak through the right or left spiracle, or through both. Likewise *Diploptera* squirts noxious quinones at predators through spiracle 4 on whichever side is attacked (Roth & Stay, 1958). The morphology and activity of spiracles 4, 5 and 9 of *Blaberus* are like those of *Diploptera*, but asymmetrical activity seems to be most strongly developed in the non-glandular spiracle 10. Another function for this behaviour may be postulated. Spiracle 10 opens very close to the cercus and each expiratory blast of air from the valve sets up a barrage of sensory impulses which have been recorded in the cercal nerve. Sealing the spiracle reduces the response; it is also diminished greatly when the spiracle is coupled to inspiration and serves an inhalant function. Repeated excitation of the cercal nerve during expiratory coupling may bring about habituation of some central synapses (Hughes, 1965). In *Periplaneta* each cercal nerve synapses with ipsi- and contralateral giant axons (Parnas & Dagan, 1971). Excitation of one cercus in *Blaberus* therefore probably leaves many synapses supplied from the other in a state of readiness to transmit. However, since cerci are moveable the amount of stimulation they receive during expiration in an unrestrained cockroach is unknown. Moreover, the dorsal surface of the cercus, which is the part mainly affected by expiration, has many fewer sensilla on it than the ventral surface.

Rowell (1969, 1971*a, b*) has argued that differing states of arousal in locusts are detectable and that they may be partly affected by sensory input. A 'dynamogenic' or arousing function for sensory input, in addition to the transmission of specific information, has many times been postulated (e.g. Bayramôglu-Ergene, 1966; Dethier, 1969; Hoyle, 1970) and it is possible that many activities such as cleaning, grooming, haltere whirring before flight and warm-up behaviour may serve the additional function of arousal through an increase in sensory activity. However, the suggestion that *Blaberus* operates a system of self-arousal by blowing on its cerci does not yet seem to be justified by the evidence.

SUMMARY

1. Spiracles 10 of *Blaberus giganteus* can be coupled with ventilation so as to open either with inspiration or with expiration. Similar activity in a mantid has been shown to produce either anteriorly or posteriorly directed ventilating airstreams in longitudinal tracheae. Switching between the two modes of coupling, which can be controlled experimentally, may pass through a maintained transitional stage when both forms occur.

2. Coupling with inspiration involves symmetrical activity in opener muscles of left and right spiracles. However, when coupled to expiration only one spiracle is active. The unilateral application of electrical or mechanical stimuli to various regions of the body causes the spiracle on that side to open with expiration.

3. Medially divided abdominal ganglia still joined to the CNS through the connectives are capable of both forms of coupling. It is suggested therefore that reciprocal inhibitory coupling between coordinating interneurons and not between motor neurons accounts for asymmetrical activity when the spiracles are coupled to expiration.

4. Spiracles 3, 6, 7 and 8 act in a similar way to spiracle 10 but have higher thresholds. Spiracles 4, 5 and 9 are associated with glandular structures and their activity is different. They may allow chemicals to be expelled through them when the insect is strongly stimulated.

5. A model is presented which accounts for some aspects of the activity of spiracles 10.

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